PARASITOLOGY

(Founded by G. H. F. Nuttall, F.R.S.)

EDITED BY

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VOLUME 40, NOS. 1 & 2 JANUARY 1950

A METHOD FOR STUDYING THE DISTRIBUTION AND BIONOMICS OF TROMBICULID MITES (ACARINA: TROMBIDIIDAE)

BY B. M. JONES, *Department of Zoology, University of Edinburgh**

(With 7 Figures in the Text)

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I. INTRODUCTION

Larval mites of the genus *Trombicula* Berl. are known to be conveyors of disease in some parts of the world. Their importance in this respect became even more apparent during the war, because they were the infective agents of an acute febrile disease, commonly referred to as scrub-typhus, which occurred among the military forces in the Far East. This gave a new impetus to detailed investigations.

The family Trombidiidae includes five other larval genera—*Eutrombicula* Ewing, *Schongastia* Oudms., *Neoschongastia* Ewing, *Gahrliepia* Oudms. and *Leeuwenhoekia* Oudms.—species of which have been found to attack man, but from a review of the literature it may be inferred that, so far, only species of *Trombicula* are proven vectors of mite-borne typhus.

Most attention has been devoted to the study of trombiculid mites in the East, because it was known that the Japanese river fever or tsutsugamushi disease was due to the larvae of *T. akamushi* Brumpt. transmitting the causative organism *Rickettsia orientalis*, from rodent to man. From recent studies of mite-borne typhus in New Guinea and adjacent islands, it has been concluded that the tsutsugamushi disease occurs, with some variations in clinical manifestations, in many parts of the Far East and has been recorded under other names, such as scrub-typhus and rural typhus in south-east Asia, adjacent islands of the Indian Ocean and the south-western Pacific and northern Queensland. At least two species, *Trombicula akamushi* and *T. deliensis* Walch, are proven vectors of the disease in the East, although others are suspect.

Keay (1937) gives an account of mite-borne diseases in different parts of the world. In Denmark, a disease called 'August Kneider', which occurs every year, is caused by *T. autumnalis* Shaw (Portchinsky 1914), a species found widely distributed in the British Isles. A kind of typhus also occurs in Mexico and is transmitted by *T. tlazahuate* Brumpt., a species which is probably identical with the American species *T. irritans* Riley (Ewing, 1932).

Those species which are not vectors of disease, but which attack man, can be serious pests, since they cause intense irritation at the site where the larval mite injects its secretion, which is responsible for the skin reaction. *T. autumnalis*, for example, has a higher nuisance value than is realized in the British Isles, where the larval mites are commonly known as 'Harvest-bugs' or 'Berry-bugs'. A survey (to be

* The author served in the Far East with the Medical Department of the Navy during the war.

published), on similar lines to that of Keay (1937), which I made for the counties around Edinburgh to determine the distribution and nuisance value of *T. autumnalis* for 1947, confirms this statement. Moreover, it may be said as a result of this survey that the medical importance of this pest has not received the attention it deserves, and it is surprising that so few observations have been published.

The importance of trombiculid mites* in agriculture is no less interesting. They are serious pests on poultry and frequently cause the death of young chickens. Gill, Moule and Rick (1945) attributed the cause of trombidiosis of sheep in Queensland to *T. sarcina* Wom.

In view of the scattered and incomplete information on the distribution and bionomics of trombiculid mites the chief aim of the present paper has been to apply a light-trap of simple design to an ecological study of *T. autumnalis*, with comments on some of the intriguing problems associated with these mites in the field. This investigation was primarily incited by a necessity to acquire at least a desired knowledge of the mite in its natural environment before designing laboratory experiments for assessing the value of its sensory perceptions with respect to the problem of acquiring a host. On completion it is hoped to publish the results of this work on the sensory physiology of *T. autumnalis*. But here and there in the text of the present paper references have been made to results of preliminary laboratory work related to the responses of the mite to stimuli it will encounter in the field.

II. METHODS USED FOR ASSESSING THE DISTRIBUTION OF TROMBICULID MITES

During the war the medical importance of trombiculid mites in the East led to a more detailed investigation of their habits and distribution. Although these mites were proven vectors of scrub-typhus in the East—invaluable knowledge in itself—an entirely new situation had arisen. Armies of non-immune men were moving through areas with high endemicity and were being attacked by disease-carrying mites, the habits and distribution of which were little known. The main problem facing investigators was how to determine the kind of habitat favoured by the mites. The methods adopted for this purpose may conveniently be classified as 'direct' and 'indirect', and workers relied principally upon the use of indirect methods for assessing distribution, particularly during the early phases of the investigations.

(1) *Indirect methods*

(*a*) *Deducing distribution from manifestations of disease*

Should a high incidence rate of scrub-typhus be recorded in a particular area it was deduced that it provided the kind of habitat favoured by the mites. Thus it was found that probable habitats were poorly drained wet areas, covered by a growth of tall, coarse grass, called Kunai grass. Emphasis was also laid on low scrub (forest) of cut-over jungle, banana and coconut groves, etc., and the list of habitats apparently favoured by the mites lengthened.

Preventive measures, based on this indirect evidence, were laid down. Poorly drained areas were to be avoided. Grass shrubbery and fallen leaves were to be removed, and advice was given to keep to prepared trails and roads when practicable.

(*b*) *Correlating distribution with the presence of rodents*

It was known that mammals and birds were hosts of trombiculid mites. Examination of rodents in areas associated with scrub-typhus showed that they were infected with the vector species of mites. In the light of this evidence, emphasis was eventually laid upon the extent of the rodent population, rather than upon a particular kind of countryside, as an indication of the presence or abundance of mites.

Consequently it was considered that areas with a heavy rodent infection should be avoided, but the difficulty of assessing the extent of the rodent population in any area can easily be imagined. This indirect method certainly could not give a true indication of the distribution of mites, because rodents were found to occur everywhere.

(2) *Direct methods*

(*a*) *Boot collections*

This method may not be regarded as suitable for working out in detail the distribution of mites in an area, but it was at least an attempt to determine the typical habitat by collecting the mites directly from the soil.

The collector stood still in situations likely to harbour mites and gathered them from his boots by means of a camel-hair brush moistened with alcohol.

Investigations in Queensland and New Guinea for assessing the distribution of the mites by this method showed that they did not occur generally in scrub or in association with rotting logs, as was popularly believed. They were found along the edge of the scrub within a belt a few yards wide. Typical habitats were tracks and small clearings in the scrub where grass grew weakly or not at all, and the ground under trees or bushes in open savannah or cleared scrub. Kohls, Armbrust, Irons & Philip (1945) also stated

* In the text, the term mite refers to the larval stage unless otherwise stated.

that the connexion between Kunai grass and tsutsugamushi disease had been over-emphasized.

Thus a reversal in ideas with regard to typical habitats was brought about by the use of a direct method. McCulloch (1946) observed that there was a great difference in the species distribution as indicated by collections from animals and from boots.

The boot method has decided disadvantages. It is a slow process because the investigator has to stand still for a period of time on each site chosen. It is not surprising, therefore, that Philip & Kohls (1945) and McCulloch (1946) stated that only small numbers of vector mites were taken from boots, whilst they were abundant on rats. This difference is understandable in view of the limitations of the boot-collecting method and the fact that rats accumulate mites when they pass through numerous pockets of infection.

(*b*) *Visual search*

This method, adopted by some investigators in order to make an intimate study of the habits of the mites upon surface vegetation, was inadequate for assessing distribution. It depends upon exceptionally careful observation in the field, and when dry conditions prevail the possibility of finding solitary mites is exceedingly remote.

III. REFERENCE TO THE STUDIES ON *TROMBICULA AUTUMNALIS* SHAW

Buxton (1945), referring to scrub-typhus, thought that the study of *T. autumnalis* in this country would throw light on the acarological side, and that, by applying methods worked out for problems of soil entomology, much might be learned about the biology of the animal.

A review of the literature shows that our knowledge of this common mite is fragmentary. Workers have collected *T. autumnalis* either by diligent visual search for clusters of these mites, or by removing them from mammal and bird hosts.

Brandis (1897), Bruyant (1910), Hirst (1926), André (1930) and Keay (1937) seem to have relied principally on collecting fed larvae from mammals and birds for their successful breeding of the larva to the nymphal stage. Elton & Keay (1936) obtained a large collection of mites from bank voles trapped over a period of three years.

André (1930) gives a list of animals upon which the mites are found on the continent, and Keay (1937) does the same for the British Isles with additional notes on the choice of host habitats. The most usual habitat is inside the ears of dogs, cats, rabbits and voles, but, if the parasitism is high, they are to be found on other parts, and cats and dogs, for example, may be badly attacked between the digits. In the case of the wood mouse and the sparrow, the usual habitat is around the anus.

The sporadic distribution of the mites is puzzling, as even one habitat may be heavily infected whilst an adjacent habitat, apparently similar in every respect, may be entirely free of mites. In a broader sense, it is interesting that in France the mites appear from observation to show a preference for a clay soil, whilst in the British Isles a chalk soil is favoured.

The lists of hosts and indirect ecological surveys which have been made are a valuable contribution to our knowledge, but still little is known about the precise habits and distribution of the mites during their free-living existence prior to infecting a host.

It seemed, therefore, that an understanding of this problem and, moreover, an insight into the behaviour of the mites under natural environmental conditions, depended upon an efficient technique for directly assessing their distribution upon the soil surface.

IV. THE LIGHT-TRAP METHOD

In June 1945 I was using a light-trap for collecting the culicine mosquitoes, *Culex gelidus* Theo., which rest during the day upon brushwood and debris in gardens of domestic buildings in Colombo. The fact that mites were collected in the mosquito trap did not impress me at the time because it was incidental to the need for mosquitoes.

At the end of 1945 the light-trap was tested for the collection of mites. It was not very successful, probably because at the time: (*a*) too much attention was paid to correlating dead leaves and debris with the presence of mites, and (*b*) it was not appreciated that a single trap used at random depends too much upon chance for detecting a concentration of mites.

During the summer of 1947 trombiculid mites became a nuisance in the Colinton area of Edinburgh, and an opportunity was provided for experimenting with the light-trap for collecting the mites directly from the soil surface.

The soil-washing technique was also tried at first, but it was quickly abandoned when it was found that the slightly modified mosquito light-trap, which I tentatively used in 1945, was successful for collecting large numbers of mites. Success was immediate because some of the traps were placed directly upon sites harbouring concentrations of mites.

(1) *The light-trap*

Apart from its unfailing efficiency to detect mites living on the surface of the soil, the light-trap has the all-important practical virtue of being simple in design and easily transportable. Forty to fifty traps, and accessories, can be carried easily by an investigator.

(*a*) *Materials*

The materials required are celluloid; adhesive tape; paper, which is waterproof, lightproof and thick-textured, and preferably of a colour to contrast with the reddish colour of the mites. For this purpose black, blue or white provide a good contrasting background, but dark grey paper is also suitable.

(*b*) *Design*

In a square of lightproof paper approximately 2 ft. sq. a hole 3 in. square is cut out of the centre. A celluloid window 3¼ in. square is placed over the central square hole in the paper, so that it overlaps the edge on all sides. The celluloid window is then firmly attached to the paper on both sides with suitable lengths of the adhesive strips, which form the frame of the window which is about 2¾ in. sq.

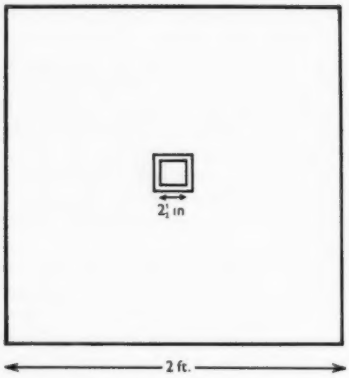


Fig. 1. A plan of the light-trap.

facilitate the method of collecting and the counting of the mites.

(2) *Technique*

The light-trap is laid upon the site selected. Provided both sides of the trap give the same colour contrast with the reddish mites, it is immaterial which side faces the substratum. The edges of the trap are then battened down by placing strips of wood, bricks, stones or any suitable material that may be available, along the edges.

Alternatively, holes may be pierced along the edges (each hole may be reinforced with strips of adhesive paper) which are then held down by pegs pushed through the holes.

When the trap is in position, the inner surface is in contact with the surface soil or surface vegetation, and it is preferable to allow contact also with the celluloid window, for reasons given later. As far as possible light must be excluded at the edges, and the only light entering the 'chamber' formed between the trap and the substratum should be through the celluloid window. About 15 min. after the trap has been placed upon a site positive for mites, there is no difficulty in detecting the reddish mites moving actively, dorsal side down, upon the inner surface of the window. If the trap is kept in position for an hour or more, the number of mites increases because they remain upon the inner surface of the window. The natural tendency of the mites to cluster may also be observed when they are allowed to accumulate in this way.

It is preferable, either when collecting material for identification or, even more important, when making counts, to examine the trap after an initial period of 30 min. or less, to prevent the number of mites on the window becoming excessive. Thereafter

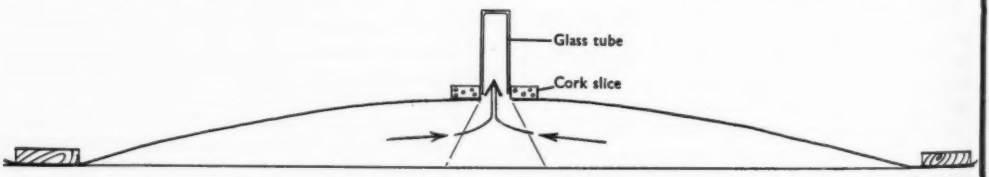


Fig. 2. The mosquito light-trap.

The mosquito light-trap (Fig. 2) was similar in design, except that a slice of cork with a central hole was gummed to the paper so that the hole in the cork was superimposed upon the hole in the paper. The cork slice carried an inverted glass specimen tube. The mosquitoes, attracted to the light source, were trapped in the tube which could be removed and replaced by another.

The slight modification of the mosquito trap simplified the design, but it was made principally to

successive examinations may be made according to the number of mites being recorded.

Accessory materials essential for the actual collecting and counting are a fine camel-hair brush, a wide-necked, glass-stoppered jar of suitable size (250 c.c.), a container to hold water for moistening the brush, and filter-papers or blotting paper.

The procedure of collecting, counting and transferring the mites from the trap to the glass-stoppered jar requires certain precautionary measures, because

the mites are incredibly active and possess the most extraordinary powers of escaping from a storage container. The following technique is therefore recommended:

(1) Cut up some filter-paper into small pieces that can be conveniently dropped through the neck of the glass jar. Moisten the pieces of filter-paper with water and place them upon a filter-paper circle.

(2) Wet thoroughly, with the brush, the inner surface of the wide neck of the jar. The water runs down and forms a wet band round the base of the neck. This retains the mites, which show a strong tendency to crawl up the sides of the jar, but on every occasion the stopper should be removed only for the shortest possible time.

(3) Remove the weights or pegs; then, by holding the two corners of the trap nearest to you, turn it over completely. The mites which are upon the celluloid window during this process are now congregated, dorsal side up, upon the reversed surface of the trap.

(4) Moisten the brush with water and count the mites as they are picked up individually. They adhere very easily to a wet brush and can be collected very quickly. It is advisable not to attempt to collect more than about thirty at a time, because, when the brush becomes overcrowded, some mites will crawl on to the wood handle and the collector's hand.

(5) Transfer the mites to one of the pieces of moist filter-paper and drop it quickly into the glass jar before the mites become active.

(6) The inside of the collecting jar, saturated with moisture, provides conditions in which the mites will remain alive for several days. The fact that practically all the mites may be retained in droplets of water does not impair their survival if living material is required.

(3) *Number of traps required*

The light-traps will presumably be used either for the sole purpose of collecting material for identification or for an ecological survey of an extensive habitat in which the distribution of mites would be measured on a comparative basis.

In the former case only about ten traps would be needed. In a confined area where mites are known to occur, it is advisable to make some preliminary tests on parts of the soil surface which are protected for the greater part of the day from direct sunlight. Shaded areas which are moist, or at least where the air humidity at the surface of the soil would be high, are likely habitats. The presence of springtails (*Collembola*) in the surface layer of the soil is also a useful indicator of the type of ecoclimatic conditions favoured by the mites. By this trial and error method in an area of approximately an acre, the light-traps will soon detect concentrated pockets of mites.

For carrying out an ecological survey an unlimited number of traps may be used if materials and workers are available. A convenient number is 100–150 traps to the acre.

A detailed investigation of an extensive area takes time because the distribution of the mites is assessed by comparative measurement based on the number of mites recorded for each positive trap over a standard period of time. Should a trap remain negative after 1 hr. has elapsed, the site can be regarded as free of mites and the trap may be moved to an adjacent site within a radius of 2 yards.

V. REACTION OF THE MITES TO THE LIGHT SOURCE OF THE TRAP

Experiments carried out in the laboratory on the reaction of the mites to light have shown that the larvae of *Trombicula autumnalis* are photopositive. The nature of this response will be published later.

A trap laid upon low-lying vegetation has its undersurface in contact with the vegetation. The mites on the soil below the trap could take an unlimited number of upward paths by way of the vegetation to reach the window. If the mites were to crawl directly upwards upon the vegetation from points on the soil where they happened to be at the time when the trap was placed in position, many of them would first reach the paper surface, before making their way towards the window. Were this the case, the expectation would be that, when the trap was reversed, mites would be found distributed at random upon the paper surface; but on every occasion when a trap positive for mites was examined, the mites were confined to the window. Close examination through the window will often reveal the mites crawling up the vegetation in the light field. The paths of the mites were observed as they wandered over the inner surface of the window, and it was noticable that a mite which wandered off the edge of the window immediately returned.

These observations suggested that the mites, in order to reach the window, crawled up only the vegetation in the light field. An experiment was carried out in the field to confirm this suggestion. A trap was laid upon a positive site, and a small stone placed under the trap directly in line with the centre of the window. Two other smaller stones marked the periphery of the light field upon the soil.

After a period of 30 min., during which the number of mites upon the window had steadily increased, the trap was removed. The surface vegetation, within the light field, marked by the stones (*A*, see Fig. 3) was cut quickly and carefully with scissors about $\frac{1}{4}$ in. from the soil (*C*, Fig. 3). The cut vegetation was transferred to a bowl of water. Within the dark field

(*B*, Fig. 3) two areas of surface vegetation, each approximately equivalent to that of the light field, *A*, were cut in the same way and transferred to separate bowls of water.

The vegetation was thoroughly washed and the mites on it floated to the surface of the water.

The results of comparative counts are given in Table 1, which shows that mites were recovered only from the vegetation taken from the light field.

Table 1. *Comparative results showing numbers of mites found on vegetation taken from different sections within the 'chamber' of the light-trap (see Fig. 3)*

Experiment	Light field (*A*)	Dark field (*B*)	
		1	2
1	6	0	0
2	8	0	0
3	5	1	0

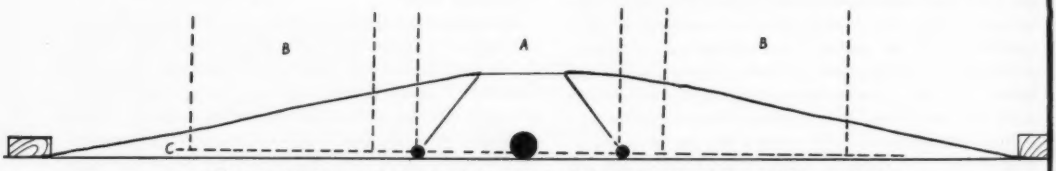


Fig. 3. Sections within the 'chamber' of the light-trap from which vegetation was removed and examined for mites.

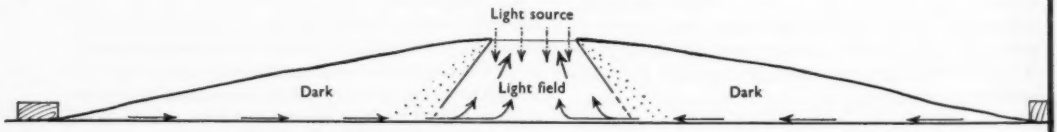


Fig. 4. Paths taken by *Trombicula autumnalis* inside the 'chamber' of the light-trap.

It is concluded from these observations that the mites in the dark field within the 'chamber' of the trap are initially attracted to the light field which they reach by crawling along the substratum. When they have entered the light field they move towards the light source and make their way up the vegetation within the light field to the under-surface of the window.

It was also significant that a light-trap was able to exhaust, in time, a concentrated pocket of mites which was roughly estimated to have the same area as the trap itself (see *A* 3, Table 2). In view of this reaction of the mites to the light-trap, it is recommended, as stated previously, that at least the celluloid window of the trap should be in contact with the soil or the surface vegetation, so that the mites may be able to reach the surface of the window directly.

VI. ECOLOGICAL SURVEY AND BIONOMICS OF *TROMBICULA AUTUMNALIS*

A large garden, measuring approximately one-third of an acre, in the Colinton district of Edinburgh, was chosen as the site of investigation for the following reasons:

(1) The incidence of mites appeared to be high, judging by their nuisance value.

(2) In a garden there is a great variety of habitats for small animals, and such habitats vary greatly from one another in physical conditions. The temperature and air humidity of ecoclimates close to the soil vary from point to point. With this in mind, it was thought that an ecological survey of mites in a garden would lend itself to an understanding of their habits and distribution and the kind of micro- or ecoclimates they prefer.

(3) Experience of the time required for examining positive traps indicated that a detailed survey of a garden measuring one-third of an acre was a suitable size for one investigator.

(1) *Description of the site*

The length of the garden was practically along a north to south line. Apart from being a convenient size with a good growth of trees, the garden contained a separate natural wooded area. This miniature wood divided the garden into two almost equal halves. The half to the north side of the wood was a level flower garden with lawns and trees. The other half was the vegetable and fruit garden which led down at a steep gradient to a level path at the foot. This level path was shaded and moist because it was the catchment area for the water draining from the steep part. A sparse hedge upon a loose-earthed bank formed the boundary between the level path and

another very steep gradient which bordered a lane below.

On the other side of this lane were riding stables which harboured rats.

(2) *Arrangement of the light-traps*

For the purpose of setting out the traps the garden was divided up into areas *A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*, *K*, *L*, *M* (Fig. 6) which could be regarded as broadly distinguishable habitats. Initial tests with light-traps were carried out to obtain a general idea of the distribution of mites in the garden. According to the initial findings a number of traps was then placed within each area (see Fig. 6). When the survey had settled down, it was possible to move

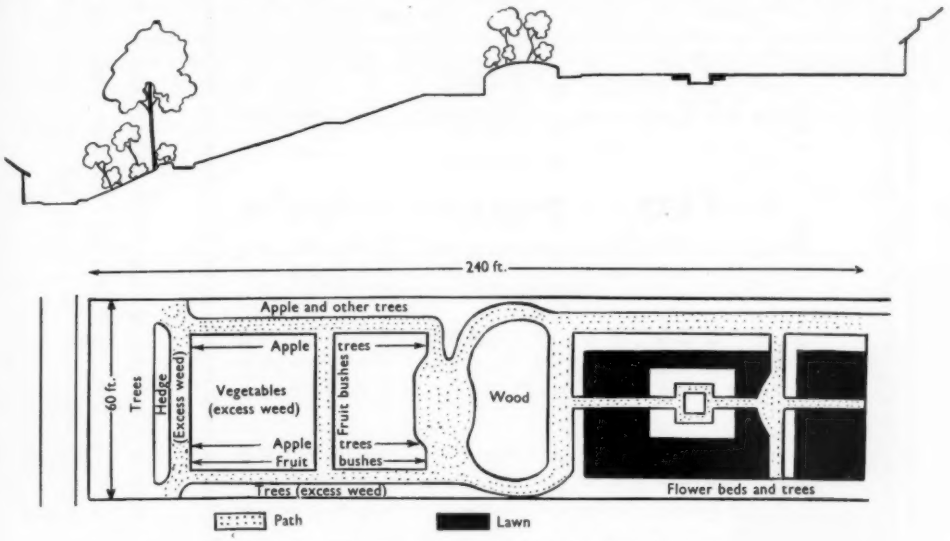


Fig. 5. A plan and section of the site of the ecological survey.

negative traps to adjacent sites within a reasonable radius of a few yards of the original site chosen. This procedure was carried out to ensure that no concentration of mites in the near vicinity of the original site was overlooked.

(3) *Recording physical conditions*

During the survey, which lasted from 21 August to 21 October 1947, the weather conditions, except for October, were unusually constant for long periods. The recordings of temperature and air humidity for the ecoclimatic conditions of each site were more important from the point of view of the survey. To record temperature thermometers were placed on the surface of the soil. Hygrometers were used for recording the air humidity close to the substratum. Samples of the surface soil were collected in clean stopped specimen tubes and taken to the laboratory for pH determinations.

VII. RESULTS

Seventeen of the forty-seven sites examined were positive for mites. Fortunately, the positive sites were confined to two areas which facilitated examination.

The number of mites collected for each positive trap were those collected at intervals for a standard time of 60 min. The traps were set out in rotation and, when the last trap was laid, the first was ready for examination. To complete a day's detailed survey of this area, measuring a third of an acre, it required not less than three, and usually an average of four, hours' continuous work for one investigator.

It was observed that during the morning survey on 22 August the reaction of the mites to the light-trap was slower than it had been during the previous afternoon. A comparison of the figures for the positive sites (A^3, B^1, B^2, B^3) on 22 August with those for 21 August illustrate the slower reaction (see Table 2).

The effect of temperature upon mites in tubes indicated that they were very active at the laboratory temperature of 20° C. and even more active at 25° C. in the controlled temperature room. Mites in a tube held near an open refrigerator were much less active at 15° C., and were motionless at 10° C., but recovered when kept at 20° C. in the laboratory.

Table 2. *Total numbers of mites* (Trombicula autumnalis *Shaw*) *collected at the end of* 15, 30 *and* 60 *min. periods of time during investigations carried out in August, September and October*

Site* (see Fig. 6)	Average figures for physical conditions recorded between 21 Aug. and 12 Sept. T° C.	% R.H.	pH	Date ...	21 Aug.			22 Aug.			26 Aug.			29 Aug.			1 Sept.			5 Sept.			12 Sept.			21 Oct.				Total nos of mites collected during the survey
				Time of day ...	p.m.			a.m.			p.m.			p.m.			p.m.			p.m.			p.m.			p.m.				
				Time in min. ...	15	30	60	15	30	60	15	30	60	15	30	60	15	30	60	15	30	60	15	30	60	15	30	60	T° C.	
A^3	17·8	86	6·3		1	1	**1**	0	1	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		3
A^3	16·7	82	6·4		32	52	**64**	10	26	**39**	16	26	**41**	.	32	**46**	0	5	**12**	0	0	**1**	0	0	0	0	0	0		204
B^1	18·3	82	6·6		14	23	**33**	4	6	**14**	6	11	**19**	6	1	**16**	.	13	**18**	.	2	**10**	2	2	**4**	0	0	0	7·2	114
B^2	17·8	81	6·8		10	16	**30**	6	9	**20**	10	14	**23**	2	6	**14**	.	3	**11**	.	3	**9**	4	10	**11**	0	0	0		118
B^3	17·8	82	6·6		16	26	**38**	6	18	**26**	4	7	**9**	6	9	**19**	.	10	**24**	.	4	**12**	6	8	**12**	0	0	0		140
C^3	17·2	80	6·4		0	1	**1**	0	0	0	2	4	**4**	0	0	0	.	5	**7**	.	4	**6**	1	1	1	0	0	0	8·3	19
D^1	21·1	73	6·7		0	0	0	0	0	0	0	0	0	0	0	0	.	2	**3**	.	2	**6**	0	0	0	0	0	0		9
E^1	16·7	80	6·9		.	.	.	.	.	.	.	.	.	.	60	**104**	70	100	**146**	.	35	**63**	0	2	**4**	0	0	0		317
E^3	18·9	77	6·4		.	.	.	.	.	.	1	4	**4**	2	8	**9**	0	0	0	.	7	**7**	0	0	0	0	0	0		20
E^4	17·8	82	6·9		.	.	.	.	.	.	.	.	.	.	72	**100**	64	98	**142**	.	30	**56**	30	40	**52**	0	0	0		350
E^5	17·8	80	6·7		.	.	.	.	.	.	.	.	.	17	25	**35**	19	23	**35**	.	76	**118**	20	36	**50**	.	.	.	8·3	251
E^6	18·3	79	6·8		.	.	.	.	.	.	20	46	**64**	35	51	**63**	16	24	**47**	.	26	**38**	.	5	**7**	0	0	0		219
E^7	17·2	79	6·9		.	.	.	.	.	.	32	58	**80**	.	76	**94**	64	90	**122**	.	20	**30**	3	9	**9**	0	0	0		335
E^8	18·9	76	7·0		.	.	.	.	.	.	.	.	.	1	1	**3**	0	1	**7**	.	0	**0**	0	0	**0**	0	0	0		10
E^9	18·3	77	6·5		.	.	.	.	.	.	.	.	.	0	0	**0**	5	8	**9**	.	0	**0**	0	0	**0**	0	0	0		9
F^1	.	.	.		0	0	**0**	0	0	**1**	0	0	**0**	0	0	**0**	.	.	.	0	0	**0**	0	0	**0**	0	0	0		1
F^2	.	.	.		0	1	**2**	0	0	**0**	0	0	**0**	0	0	**0**	.	.	.	0	0	**0**	0	0	**0**	0	0	0	7·8	2
F^3	.	.	.		0	0	**0**	0	2	**2**	0	0	**0**	0	1	**1**	.	.	.	0	0	**0**	0	0	**0**	0	0	0		3
G^1	19·4	80	6·4		0	2	**8**	0	0	**0**	1	3	**3**	0	0	**0**	.	1	**1**	.	0	**0**	0	0	**0**	0	0	0		12
G^2	21·1	80	6·4		0	0	**0**	0	0	**1**	0	0	**0**	0	0	**0**	.	0	**0**	.	0	**0**	0	0	**0**	0	0	0		1
H^1	21·1	80	6·6		0	0	**0**	0	0	**0**	3	5	**5**	0	0	**1**	.	0	**0**	.	0	**0**	2	2	**3**	0	0	0		9
H^4	20·0	78	6·2		6	15	**29**	8	9	**16**	16	18	**22**	6	12	**16**	.	8	**20**	.	4	**10**	6	10	**14**	0	0	0		127

* Negative sites, although examined at standard times on the above dates, are not included in this table.

Table 3. *Physical conditions recorded between* 21 *August and* 12 *September for negative sites* (*see fig.* 6) *during the ecological survey*

Site	A^1	C^1	C^3	C^4	C^5	D^2	E^2	E^{10}	E^{11}	E^{12}	F^4	G^3	G^4	G^5	H^2	H^3	H^5	H^6	K^1	K^2	L^1	L^2	M^1	M^2	M^3
Average T (°C.)	16·1	22·2	23·9	17·8	23·9	23·9	17·2	19·4	21·1	18·9	—	21·7	22·2	20·0	21·1	20·6	22·2	21·1	20·6	21·7	22·8	12·8	20·0	20·0	18·9
Average % R.H.	82	72	70	75	63	65	78	76	77	76	—	68	70	72	70	60	65	68	58	55	75	53	64	65	70
Average pH	6·2	6·7	6·3	6·2	6·0	6·9	6·2	6·4	6·5	6·5	—	—	—	—	6·3	6·4	6·4	6·4	—	—	—	—	—	—	—

In view of these observations the remaining surveys were made during the afternoon to ensure greater activity of the mites after the soil had been warmed by the high midday temperatures. Mite activity reached a peak by the late afternoon, which suggests a soil temperature-activity relationship for mites.

avoid direct tabulation of the negative findings recorded for each survey.

In order, primarily, to recover a maximum number of mites from the soil, traps were also placed at a distance of 1 ft. from the periphery of detected positive sites. It soon became apparent, and it was certainly significant, that sites within a short radius

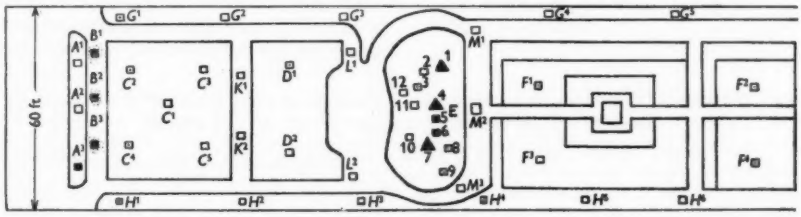


Fig. 6. The distribution of *Trombicula autumnalis* as distinct pockets of infection. Total numbers of *T. autumnalis* collected from each site. □ negative, ⊡ 1–20, ⧈ 100–200, ■ 200–300, ▲ 300–400.

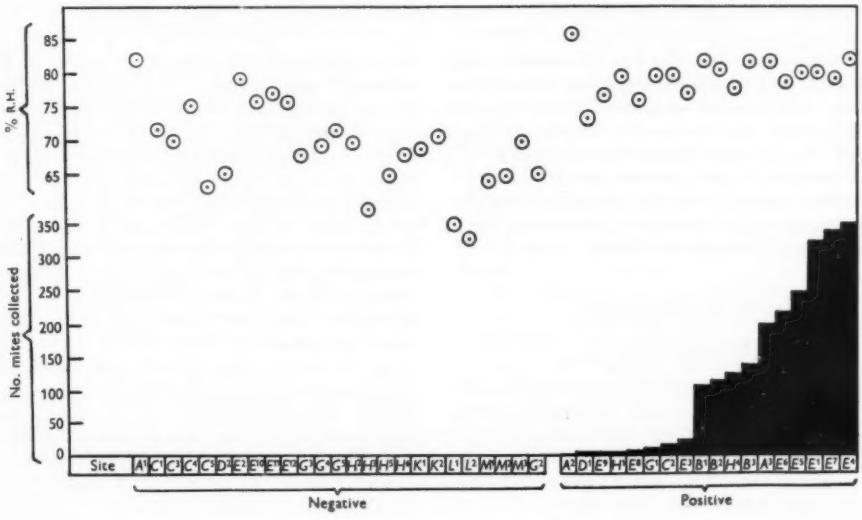


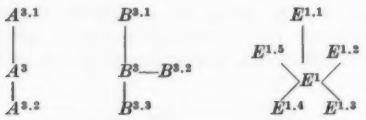
Fig. 7. Histogram showing the relationship between mite distribution and relative humidity.

The detailed results are presented in tabular form because, with reference to Fig. 6, the distribution of the mites is clearly defined. Table 2 shows the total numbers of mites recorded for positive sites at 15, 30 and 60 min. for the individual surveys, the figure in the third column for 60 min. being the gross total for each site. Negative sites, although examined during each survey, are not included in Table 2 but are presented in Table 3 with related recordings of physical conditions. This procedure was adopted to

were negative or nearly negative. This indication of mites being concentrated in pockets around a focal point was more marked in area *E*, than in area *B*, where they were more widely dispersed around the foci.

During individual surveys five traps were placed around site E^1, three around site B^3 and one on each side of site A^3. A change in the comparative numbers of mites recorded for the traps arranged around the foci of infection would indicate either a shift of the focal point, or a dispersal of the concentration.

The following arrangement of traps around the sites A^3, B^3 and E^1 are in relation to the position of each site as shown in Fig. 6:



diffused over the moist, weed-covered path (area *B*). The exception was the adjacent site (A^3) upon the loose-earthed bank where a separate pocket was detected (see Fig. 6). It was interesting that the light-trap appeared to exhaust this site completely by 1 September (see Table 2), and it was suspected that this also happened later to the mite density at sites E^1, E^6, E^7.

The characteristic manner in which mites are distributed in foci or concentrated pockets of infection, as shown by the light-trap method, explains the

Table 4. *Numbers of mites collected at the end of 60 min. from traps arranged at a distance of 1 ft. around the focal points of infection A^3, B^3, E^1 (see Fig. 5)*

Date of investigation	Focal point A^3	Surrounding sites		Focal point B^3	Surrounding sites			Focal point E^1	Surrounding sites				
		$A^{3.1}$	$A^{3.2}$		$B^{3.1}$	$B^{3.2}$	$B^{3.3}$		$E^{1.1}$	$E^{1.2}$	$E^{1.3}$	$E^{1.4}$	$E^{1.5}$
21 Aug. 1947	64	0	0	—	—	—	—	—	—	—	—	—	—
22 Aug. 1947	39	0	0	38	10	1	4	—	—	—	—	—	—
26 Aug. 1947	41	0	0	23	2	2	6	—	—	—	—	—	—
29 Aug. 1947	46	1	0	19	4	4	8	104	1	0	0	4	2
5 Sept. 1947	12	0	0	12	5	2	3	63	0	10	2	0	6
12 Sept. 1947	1	0	0	12	0	5	0	4	0	0	0	1	0

The results show that the foci remained at the sites A^3, B^3 and E^1 (Table 4). Moreover, whereas the mites were concentrated as pockets around the foci of sites A^3 and E^1, they were widely dispersed around the site B^3. Spot tests indicated that the sites B^1 and B^2 were similar to B^3, as the sites in area *E* were to E^1.

To illustrate a mite distribution-humidity relationship a histogram (Fig. 7) has been constructed from the original data presented in Tables 2 and 3.

VIII. DISCUSSION

(1) *Distribution*

A significant feature of the survey was that the mites were concentrated in two areas, the shaded, moist, weed-covered path (area *B* and adjacent sites A^3, C^1, C^3, G^1, H^1) and the wood (area *E*). The remaining sites, except for a small mound of grass debris (H^4), were practically free of mites (see Fig. 6).

In the wood (area *E*), the mites were distributed as well-defined concentrated pockets of infection. Throughout the survey these concentrated pockets (E^1, E^3, E^4, E^5, E^6, E^7) did not shift their foci, and adjacent sites within a radius of 6 ft. remained practically negative. The physical conditions for both positive and negative sites in area *E* were very similar, except that the pH for the infected sites, with one exception, was distinctly higher (see Table 3).

The other concentration of mites (area *B*) did not display so markedly this further division into separate pockets of infection. Whereas the focal points were the sites, B^1, B^2, B^3, the mites were observation that one habitat may be heavily infected whilst an adjacent habitat, within a short radius and apparently similar in every respect, may be free of mites (see area *E*, Fig. 6). It would also explain the commonly occurring case of perhaps only one member of a party showing the symptoms of an attack by mites, although all the members had been in the same locality. Reference to the distribution of mites in the wood (area *E*, Fig. 6) shows how, for example, three people could stand on three sites within a short radius, one of which was positive for mites, with the result that only one person would be attacked. The same limited attack could occur if only one member of a party stood still on a positive site in the wood whilst the other members walked straight through the positive sites. My experience shows that it is extremely unlikely that a person walking through infected sites without stopping would be attacked. It must be kept in mind, however, that some cases of only one in a party being affected by the mites may be explained by the fact that some individuals have a natural immunity. It is also true that others acquire immunity over a period of time.

(2) *Physical conditions*

It was clear from the results of laboratory investigations of the effect of humidity upon mites that they survived longest in saturated air, provided the temperature was favourable. The effect of pH upon mites indicated that the duration of life is longest within a range of pH 6·4 to 8·0. These laboratory findings, although part of preliminary

work being carried out to elucidate the behaviour of mites in relation to environmental conditions, are included here because they are relevant to the physical conditions recorded during the survey.

The field data given for temperature, humidity and pH presented in Table 2 excludes any reference to a term like 'optimum conditions' for these mites.

The pH figures for the surface soil of all the sites sampled ranged from pH 6·2 to 7·0. Of the ten infected sites with a gross total exceeding 100 mites, eight (B^1, B^2, B^3, E^1, E^{4-7}) were within the range pH 6·6–6·9, while the remaining two (A^3) and (H^4) were pH 6·4 and 6·2 respectively. These results cannot be taken to imply that pH has a significant relationship with mite density, because both negative and near-negative sites came within the range pH 6·6–6·9.

Temperature undoubtedly influences the seasonal incidence of mites, and the low soil temperatures of October in the British Isles are responsible for their disappearance from the soil (Table 2). Variations of temperature within the range 10–25° C., according to laboratory tests, only cause increased or decreased activity of the mites, but do not influence their survival at high humidities. During the mite season in the British Isles, the soil temperatures would fluctuate within this range, therefore temperature will have only an activating influence in a microhabit harbouring a density of mites.

Laboratory results and field data (Tables 2 and 3) strongly imply that humidity is the limiting factor. It was significant that the humidity recordings for the positive sites were, with few exceptions, constantly high and not below R.H. 78 %. This relationship between mite distribution and humidity is shown in the histogram (see Fig. 7).

(3) *Disappearance of the larval stage from the soil*

The mites completely disappeared from the soil in October. Spot tests indicated that they had disappeared by 14 October, and a detailed examination on October 21 verified the negative results (Table 2). The latter day was particularly warm for October, but the soil temperature of 8° C. was low. It was expected that the trap method would give negative results, because at less than 10° C. the mites become motionless. Soil samples were therefore taken, to a depth of 1 ft., from those sites which had been positive throughout the survey. The soil was thoroughly washed in water, but careful examination with a binocular lens failed to detect mites floating on the surface of the water.

This method of examining soil samples or tussocks of grass, used as a subsidiary one in the survey of 21 October, is widely applied for working out problems of soil entomology. Usually samples are taken at set distances but sometimes at random, within an area. Statistics for population, seasonal incidence, etc., for the particular fauna being studied, are then based on the results of the examinations of the samples taken.

It is clear that owing to the sporadic distribution of the mites (Fig. 6) the adoption of a soil-sampling method of this kind for assessing their distribution and seasonal incidence could only lead to fallacious results.

(4) *Over-wintering*

The question of whether the winter is passed in the larval, nymphal or adult stage is an interesting one. The occurrence of larvae upon the host during the winter is responsible for the theory (Elton & Keay, 1936) that the larval stage over-winters; the adults appear in June and July, lay eggs which hatch in August, and so give rise to the new population of larvae.

In October there was no trace of the free-living larval stage in the soil. Moreover, on 12 September, two nymphs were recovered from the soil at a depth of 9–12 in.

Breeding experiments carried out in the laboratory showed that larvae fed on rabbits had fully engorged after 3–5 days. On recovery from the host they were introduced into moist glass cells. No difficulty was experienced in rearing fully fed larvae to the nymphal stage at ordinary room temperatures. The average time required for this to take place was 33–35 days, and the nymphal stages emerged in September and October.

The recovery of nymphs from the soil in September and since, at least under laboratory conditions, it seems normal for the nymphal stage to appear in October, suggest that both the nymphal and adult stages over-winter, but that the larval stage disappears. The occurrence of larvae upon hosts in winter (Keay, 1937) and Bruyant (1910) appears, therefore, to be incidental to the true life cycle of *T. autumnalis*.

(5) *Association of mites with fruit bushes*

The association of mites with fruit bushes is interesting. The results of a survey, already mentioned, to determine the distribution and nuisance value of mites in and around Edinburgh in 1947, showed that approximately 50 % of the reported cases of mite incidence were associated with fruit bushes in gardens.

During the survey special attention was devoted to an examination of gooseberry and raspberry bushes. In the garden the fruit bushes, except for one row, were confined to one side (Fig. 5), but the soil where they were growing was dry and exposed

to the sun, and careful searches for mites proved negative. The mites will therefore be found in association with fruit bushes only when the conditions of the soil are favourable.

The association between fruit bushes and mite irritation may be explained by the fact that fruit picking entails standing still for long periods on the same site, and this provides the mites with opportunities to attack. So, although mites may be distributed over other parts of the garden, they will appear to be most abundant among fruit bushes. Another explanation is that whilst birds infected with mites are feeding on the fruit, engorged larvae drop off the host on to the soil and undergo metamorphosis to the nymphal stage, which changes to the adult stage to complete the life cycle. In a laboratory experiment the nymphs displayed a decided preference for saturated humid conditions. Loose dried soil was transferred to a glass cell, and six nymphs, bred from fed larvae, were placed at the centre of the surface. To one side the soil had been moistened with damp cotton-wool so that a small limited area became moist. When the nymphs wandered into the moist area they remained there, and all six were eventually recovered from the moist side. This observation supports the view that nymphs (and adults?) would remain near the site upon which the fed larvae had originally been dropped, were the moist conditions localized.

One would therefore expect that, provided the soil was sufficiently moist, the fed larvae, dropped from birds, would complete their life cycle near the fruit bushes. The adults would lay clusters of eggs, which would give rise to a new generation of larval mites in association with the fruit bushes. Naturally, fed larvae are dropped off the host at random, and they will survive anywhere in a garden, field or wood, provided the soil conditions are favourable, but usually strawberry beds and soil sheltered by fruit bushes provide suitable habitats.

Apart from the interesting bionomic aspect, the question is important economically, because numerous cases have been reported of large-scale fruit picking being suspended on account of the attacks of the mites and the extreme irritation which followed.

(6) *Clustering*

The phenomenon of clustering, provided the temperature is favourable and the humidity is high, seems to be primarily a tactile response, the mites being responsive to contact with each others' bodies. The entanglement of the comparatively long body hairs of adjacent mites also appears to play a part in clustering. Clustering occurred frequently on the window of the light-trap, especially when condensation on the window indicated that saturated conditions prevailed in the 'chamber'. Clusters were sometimes formed on the edges of strips of filter-paper when they were placed on infected soil and left overnight. Mites kept on saturated soil in tubes also showed a tendency to cluster, and it was noticeable that they often aggregated upon the uppermost part of the surface of the soil.

According to the literature the appearance of orange clusters of these mites in the field is not uncommon, but they were not observed under natural conditions during my investigations in the dry summer of 1947. Therefore the chance of finding the orange patches of mites on the soil or upon vegetation appears to be exceedingly remote, if the conditions are unfavourable. I hope to include further observations on this question of clustering in the proposed paper on the behaviour of these mites.

IX. CONCLUSION

In the same way that the use of classical methods has contributed to our knowledge of the habits of the disease-carrying mosquito, so the light-trap method can be used in the study of trombiculid mites in relation to mite-borne disease.

This direct method of assessing the distribution of mites in an area has a distinct advantage over the methods previously adopted. It introduces a new approach to the problem of surveying an area in order to diagnose mite infection; a unit equipped with light-traps, could make a survey to locate and undertake the identification of mites in much the same way as is done for mosquitoes.

The efficiency of the method in working out, if required, the detailed distribution of foci of infection throws light on the more intimate habits of the mites in the field; and the ability to locate concentrated pockets of mites is a valuable aid in indicating sites which could be investigated for the recovery of nymphal and adult stages which lead a hypogeal mode of existence deep down in the soil. Not least important is the ease with which the solitary larvae may be collected in the field, a great advantage in connexion with obtaining material for laboratory investigations.

SUMMARY

(1) The medical importance of trombiculid mites is indicated and reference is made to their geographical distribution.

2. Methods used by investigators for assessing the distribution of the mites in the Far East are discussed as well as methods used in the study of *Trombicula autumnalis*.

3. A detailed account is given of a light-trap and the method of using it; the reaction of the mites to the trap is discussed.

4. The results of a detailed survey are presented to show the significance of the method in relation to the distribution and bionomics of trombiculid mites. The main results of this survey are as follows:

(*a*) The mites are characteristically distributed in pockets of infection.

(*b*) A study of the ecoclimatic conditions favourable to mites shows that humidity is a limiting factor.

(*c*) The free-living larval stage disappeared from the soil in October, and nymphs were found in the soil in September. These facts, together with the results of laboratory breeding experiments at room temperatures, which showed that it was normal for nymphs to appear in October, indicate that the nymphal and adult stages over-winter.

(*d*) Mites are not associated with fruit bushes if the physical conditions of the soil are unfavourable. A tentative explanation is given to account for the frequent association of fruit bushes with mite-irritation.

(*e*) The gregarious habit of clustering is primarily due to a tactile response.

5. It is concluded that the light-trap method offers a new approach to the problem of surveying an area in order to diagnose trombiculid mite infection. It is also of value in obtaining abundant material for laboratory investigations.

My thanks are due to Prof. James Ritchie for his interest and encouraging help in this work which may be regarded as preliminary to a proposed study of the different aspects of the biology of *Trombicula autumnalis*; to Dr D. T. Gauld for helpful criticism; and to the owner of the garden who gave me complete freedom to carry out the survey.

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THE ECOLOGY OF THE SHEEP TICK, *IXODES RICINUS* L.

MICROHABITAT ECONOMY OF THE ADULT TICK

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(With 1 Figure in the Text)

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1. INTRODUCTION

In its various stages, the tick (*Ixodes ricinus* L.) spends a total of about 3 weeks on hosts; the remainder of its life, about 3 years, is passed on the ground, this time being taken up in development, quiescence and host seeking (MacLeod, 1932; Campbell, 1946). Here we shall be mainly concerned with adult-tick economy within the ground microhabitat.

A good deal of laboratory work has been done on factors influencing survival, reproduction, development and behaviour of the tick (e.g. MacLeod, 1932, 1934, 1935*a*, 1935*b*). But despite inferences and general observations, the picture of the tick within its natural microhabitat is still fairly nebulous in some important respects. For instance, although *in vitro* work strongly suggests that humidity may be a limiting factor, no precise observations of humidities met by the tick in nature have been made. The present paper attempts to fill in some of the major details of general behaviour and survival in relation to microhabitat conditions. Field behaviour of the unfed adult is amplified in another paper (Lees & Milne, in preparation); and our colleague, J. Allan Campbell at Edinburgh University, is working out the minutiae of the natural development cycle.

Ticks were laid down for observation at representative points on two Northumberland farms, Hethpool and Lordenshaws. These points included the four common vegetation types among hill grazings, namely, rough bent grass (with intermingled rushes), bracken, heather, and fine grasses of the fescue kind.

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2. MALE AND UNFED FEMALE TICKS IN A DEEP VEGETATION LAYER

All unfed stages climb to the tips of the vegetation (grass, rushes, bracken, heather, etc.) when 'active'. From there they will clutch at a passing host. The active periods for any individual are comparatively few and short, e.g. the female failing to achieve a host has commonly about five periods, averaging 4–5 days each, spread over the 3-month activity season. By far the major part of the unfed tick's life is spent at some lower level in the vegetation. These facts emerge from unpublished work (Lees & Milne). Olenev (1927), from experimenting with *I. ricinus* in flowerpots containing turves from 'forest pastures' in Russia, states that unfed female ticks 'frequently buried themselves in the mossy cover' on the soil surface.

(i) *Vertical distribution of ticks in the microhabitat*

(a) *Methods*

Ticks were laid down on pasture land and, after an appropriate interval, their vertical distribution was ascertained by lifting and examining the turves containing them.

Males and unfed females for observation were developed from engorged nymphs 'rubbed' off, therefore ready to fall from, the faces and ears of sheep. Development took place in an incubator at 20–23° C. and was complete within 2 months. After metamorphosis and until set down in the field, the adults sojourned in a cool, semi-dark room at 100% R.H.

As a rule, twenty male and twenty female ticks were laid down together at a selected point. The turf lifted was a square of 8 in. side (20·3 cm.), having the original laying point at the intersection of its diagonals.

Turves were lifted at various intervals after the laying down of ticks, at times well distributed throughout the year. Details of collection of nymphs, laying down of adults and lifting of turves are given in the Appendix.

Before lifting a turf, the herbage was thoroughly brushed twenty times with the hand in order to collect active ticks. The turf was placed in a tin box, 1-2 oz. crude chloroform being poured over it before the lid was sealed. After 24 hr. the turf was sectioned into layers parallel with the soil surface. The chloroform was supposed to kill the ticks *in situ* within the turf, the method being suggested by our colleague, J. Allan Campbell. The tick distribution results so obtained, however, did not differ from those by the writer's original procedure without chloroform, namely, speedy sectioning immediately after lifting the turf, the sections being boxed separately.

Details of the turf layers are given in paragraphs (*b*), (*c*) and (*d*) below. Each layer was teased out by hand and washed through a bank of sieves by a strong jet of water. Ticks were recovered from the residue on the finest sieve by the familiar flotation-separation method using aqueous magnesium sulphate solution (1·18 sp.gr.) and Xylol. The method is very efficient for adult ticks. This was proved by introducing numbers of test ticks at different stages of the process, unknown to the operator; these ticks were all recovered. Further, in two cases of normal procedure, half of a female tick was found in one layer which fitted perfectly to another half recovered from the layer below. These ticks had been cut in the sectioning of the turf.

(b) *In rough bent grass*

At Hethpool the experimental grazing is of the rough bent grass type, chiefly *Nardus stricta* with some *Molinia caerulea* and *Juncus articulatus*. It has a thickish underlying mat of decaying vegetable matter over a medium clay soil slightly peaty at the surface. The vegetation layer is remarkably uniform in a broad fashion, and the points at which ticks were laid down yielded similar turves. The latter were sectioned into four layers, viz. grass, upper mat, lower mat and soil. (There are no really sharp dividing lines between these layers, but the sectioning 'lines' were as nearly as possible the same in every case. The same applies to heather and to bracken turves.) Grass was cut off at the mat surface; it was about 8 in. deep except in winter (October–February) when it varied from 4 to 2 in. deep as the stems lay over in the process of withering. Mat was about 2½ in. thick. The upper section, i.e. upper mat, was about 1½ in thick, composed of dead grasses with some moss, loosely intertwined. Lower mat was about 1 in. thick, comprised of decaying grasses, etc., in a tight tangled mass. After finding no ticks at a greater depth in preliminary trials, the soil layer was limited to the first ¾ in. below the mat. The living plant stems and roots run through mat and soil, toughening both. Table 1 shows the distribution of recovered ticks in the various layers at different times of the year.

(c) *In heather*

The heather pasture chosen at Lordenshaws yielded turves sectioned into four layers as follows: Heather, stout stems from 9 to 24 in. long. Strictly the term 'mat' is applicable only to the dead and decomposing vegetable matter under rough grass pasture, but the same term serves here for want of a better. Mat was about 2¼–2½ in. thick. Upper mat, about 1¼–1½ in. thick, was composed of a little living moss and much loose, dead, mossy-peaty material, easily broken up by hand. Lower mat, about 1 in.

thick, was a tightly woven layer of fibrous peaty material, requiring laborious teasing before fit for sieving. Soil, the usual ¾ in., was loose and sandy. As before, Table 2 shows the tick distribution.

withering (lying over) of the fronds. Mat, about 1 in. thick, was chiefly bracken litter with short grasses of the sheep's fescue type growing through it. The fine root system of the grass gives the mat a slightly

Table 1. *Vertical distribution of ticks in turves from the bent grass grazing at Hethpool between August* 1944 *and July* 1947 *inclusive*

	Grass		Upper mat		Lower mat		Soil	
Month	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
1944: August	7	6	36	41	4	14	1	0
September	0	0	16	14	0	0	1	0
October	0	0	7	15	0	0	0	0
December	0	1	9	8	4	5	0	0
1945: January*	0	0	5	18	2	0	0	0
February†	0	0	5	16	0	2	0	0
April	0	4	0	0	2	0	0	0
June	0	0	0	4	1	0	0	0
July‡	0	0	0	0	0	0	0	0
August	0	0	12	11	3	0	0	0
September	1	1	7	5	3	8	0	0
1947: June	0	0	19	16	1§	4§	0	0
July	0	0	57	54	2§	2	0	0
Totals	8	12	173	202	22	35	2	0

* A 4 in. layer of snow and ground frozen hard when this turf was lifted.

† During the month preceding the lifting of this turf, the maximum and minimum temperatures in the screen on the grazing were 51 and 8° F.

‡ A pair of larks built their nest and reared their young on the point at which this sample had been laid down. Probably the ticks attacked the birds and were eaten for their pains—see self-deticking, Milne, 1947.

§ These ticks were actually seen on the surface of the lower mat while sectioning; at other times we did not look for this.

Table 2. *Vertical distribution of ticks in turves from the heather grazing at Lordenshaws between November* 1944 *and June* 1946 *inclusive*

	Heather		Upper mat		Lower mat		Soil	
Month	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
1944: November	0	0	2	4	0	4	0	0
December	0	0	5	14	0	1	0	2
1945: January*	0	0	12	10	0	2	0	0
February	0	0	4	9	2	5	0	0
November	0	0	17	14	0	0	0	0
1946: January	0	0	13	14	0	0	0	0
June	0	0	0	0	0	0	0	0
Totals	0	0	53	65	2	12	0	2

* This turf was lifted just after the first snowfall, which had been 18 in. deep; the ground was thawing and some patches were still lying about.

(d) In bracken

The luxuriant bracken bed chosen at Lordenshaws was on dry, shallow, sandy soil overlying rock. Probably owing to the latter conditions it had much less basal litter than one would have expected. Consequently, 'mat' could not be subdivided into 'upper' and 'lower'. The turves yielded three layers as follows: Bracken naturally varied from inches to feet in depth according to the state of growth or fibrous character as it merges with the sandy soil. Again, Table 3 shows the distribution of ticks in the various layers.

(e) Conclusions

Table 4 summarizes Tables 1–3. The vertical distribution of males and females is obviously similar.

In many cases the turves were lifted when (November to early March), normally, ticks are not

active in northern England. Even during the tick season, never more than a fraction and usually only a small fraction of the adult ground-population is active at any moment (see Milne, 1945, 1946; Lees & Milne, in preparation). Hence it is not surprising that so few ticks were found in the uppermost, i.e. 'grass', 'heather' or 'bracken' layer.

The ticks in the uppermost layers were actually active, since all but one or two were taken by hand-brushing prior to turf lifting. Leaving these out of consideration, 674 out of the 679 inactive recovered ticks were in the mat layer; and, in the two cases where mat was subdivided, 493 of the 564 were in the upper mat, with only 71 in the lower. That is, 99% of inactive ticks are in the mat, and about 87% of these are in the upper portion of the mat. The latter fact, together with the last footnote to Table 1, would suggest that the 1% of inactive ticks (two males and three females) found in the $\frac{3}{4}$ in. soil layer were in crevices of the soil surface, that is, had not burrowed into the soil. Tables 1–3 show also that the average distribution of inactive ticks just described is the same throughout the year, unaffected by gross seasonal changes in microclimate.

In the foregoing turf-extraction experiments, all the males laid down were recovered only thrice, and all the females only four times, in thirty-two cases each. The lost ticks might have migrated out of the square of turf, or, since dead ticks are recovered as easily as live ones, they might have been devoured entirely or completely disintegrated.

(ii) *Horizontal (lateral) migration*

Migration was investigated by processing the 4 in. wide belt of turf surrounding the central square of 8 in. side. In ten cases, in which 200 male and 200 female ticks were laid down, the total recoveries were:

Central square	91♂♂	129♀♀
Surrounding belt	1♂	7♀♀

That is, 55% of the original ticks were recovered from the central square, and only 2% from the surrounding belt. The force of these figures is somewhat lessened, however, by the fact that in six of the ten cases some time elapsed between the lifting of the square and of the surrounding belt, the former always being taken first. During that time lapse, varying from 2 weeks to 5 months, some ticks in the surrounding belt could have died and disintegrated or been completely devoured: but the same sort of distribution was found when lifting was simultaneous, as Table 5 shows for the remaining four cases.

Recoveries from the central square and first surrounding belt were not much more than half (57%) of the ticks laid down. Could the remainder have migrated to a distance greater than 8 in.? Such a bimodal distribution of migratory faculty among individuals of the same age and condition would be most unusual in view of the 'normal' distribution common to biological variates. A second 4 in. wide belt surrounding the first was processed in two of the ten cases mentioned above: in one case no ticks were found, in the other a single female (see Table 5). Although twenty-six was the greatest number of

Table 3. *Vertical distribution of ticks in turves from a bracken bed at Lordenshaws between December* 1944 *and June* 1946 *inclusive*

	Bracken		Mat		Soil	
Month	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
1944: December	0	0	7	21	0	0
1945: January*	0	0	1	10	0	0
February	0	0	1	4	0	1
November	0	0	14	18	0	0
1946: March	0	0	14	20	0	0
June	0	0	0	0	0	0
Totals	0	0	37	73	0	1

* This turf was lifted during the second snowfall; the ground was frozen hard and a crisp 2 in. layer of snow covered all.

Table 4. *Percentages of recovered male and unfed female ticks in the various layers of the three pasture types*

Pasture type	Layers	Males (%)	Females (%)
Rough grass	'Grass'	3·9	4·8
	'Upper mat'	84·4	81·2
	'Lower mat'	10·7	14·0
	'Soil'	1·0	0·0
Heather	'Heather'	0·0	0·0
	'Upper mat'	96·4	82·3
	'Lower mat'	3·6	15·2
	'Soil'	0·0	2·5
Bracken	'Bracken'	0·0	0·0
	'Mat'	100·0	98·7
	'Soil'	0·0	1·3

indigenous females found per 100 sq.yd. of this grazing (Milne, 1946), it is not impossible that the single female recovered in the latter case was actually indigenous. As a matter of fact, an indigenous female was found in one of the central squares, twenty being laid down but twenty-one recovered (see December, Table 3).

In the course of observations on the behaviour of identifiable (paint-spotted) individuals in nature, the writer confirmed the view that few of the ticks laid down in the present experiments would have moved even as far as the first surrounding belt. Thus six unfed females and three males, living naturally in rough bent grass, were observed daily 6 days a week from 20 March to 25 June 1946. These alternated between activity at the vegetation tips and quiescence deep in the vegetation until activity stopped at the end of June. Five of them always reappeared on the same stem on which their first ascent was made. Of the remainder, three reappeared on a different stem, and one on two different stems. The distances between their original and subsequent (last) stem bases were:

♀	8·5 in.	♀	0·5 in.
♀	6·0 ,,	♂	2·5 ,,

Thus the mean lateral migration was less than 2 in. only two individuals in nine exceeding 2·5 in.

Much additional field observation (Lees & Milne) has indicated that unfed ticks have no inclination to move other than vertically up or down. If a tick appears on an isolated tall stem then all its movement is confined to that stem; but usually there are numbers of tall stems, leaning at different angles and touching here and there. A tick may ascend one side of a stem finding nothing to deflect it. On descending on the other side, it may come to a point where another stem touching offers an alternative downward route which it may take. The bases of these stems may be close or far apart. Thus the tick achieves horizontal or lateral change of position. This is a random thing so that the changes tend to cancel out, especially where the tallest stems are more or less erect.

Lateral migration, or rather, change of position, in males and unfed females in good natural conditions is therefore negligible on the average. Only a very small percentage of the ticks laid down would have left the central square of turf. Hence the recoveries from this square represent the great majority of survivors.

(iii) *Survival*

Six of the females and eleven of the males listed in Tables 1–3 were recovered dead, i.e. black, shrivelled, lacking all or most of their appendages and, in one case, part of the body wall; all were in the mat layer. The remaining 662 ticks were fresh coloured and intact, and hence presumably alive when recovered. The seventeen dead ticks are excluded from the following notes on survival.

In nature, after emergence, the length of time an adult survives without a host should depend, apart from 'disease', only on the physical suitability of the habitat and on predators. In the present work, however, the adults were (*a*) developed at unnaturally high temperatures; (*b*) stored for varying periods, as well as developed, in a more or less stable instead of the naturally fluctuating environment; and (*c*) possibly abnormally stimulated by transference from laboratory to pasture conditions, especially when this occurred outside the activity seasons. One or other, or all, of these circumstances may have influenced the duration of their survival. Hence the survival data from the turf-extraction experiments must be viewed in their slightly artificial context, the doubt being how much or how little importance to attach to the artificiality.

The wild and domestic hosts normal to the grazing (see Milne, 1948) were deprived of access to the ticks by fencing, but the normal predators were not. Table 6 shows the grouped data. The grouping masks an understandable irregularity of the raw data. For instance (extreme), after 265 days on the ground 2 in 17 males and 4 in 30 females were found alive in one experiment, but in another after 272 days the figures were 14 in 20 and 20 in 20. This variation would necessitate greatly extended observations before mortality rate could be estimated with precision. Within the first 100 days after emergence the mortality in unfed adults deprived of hosts is apparently of the order of 50%. Only two experiments covered a year; and in these no ticks were

Table 5. *Showing ticks recovered in the concentric areas covering approximately* 0–4, 4–8 *and* 8–12 *in. from the point at which twenty males and twenty unfed females were laid down in each of four experiments*

Turves A, B and C were always lifted simultaneously

	Exp. 1		Exp. 2		Exp. 3		Exp. 4		Totals	
Turves	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
A. Central square, of 8 in. side	7	15	7	18	5	18	5	10	24	61
B. Belt 4 in. wide, surrounding A	1	1	0	0	0	2	0	0	1	3
C. Belt 4 in. wide, surrounding B	—	—	—	—	—	—	0	1	0	1

found alive after 368 and 358 days respectively, although 20 males and 20 females had been laid down originally in each case.

Tables 1–3, 5 and 6 also suggest that mortality among the experimental ticks was greater in males than females. The overall recoveries were 46·5 % of the males laid down as against 59·5 % of the females. Whether this happens in nature is very doubtful. It may have arisen from one of the unnatural conditions of the experiment, namely, the opportunity for copulation. Olenev (1927) states that 'males die off soon after fertilization in spring'. In nature, adults are distributed singly and so sparsely on the ground (Milne, 1946) that only the host can bring them together. In contrast, the sexes were brought together in the present experiments; indeed some were *in copula* when laid down. However, our colleague Lees (private communication) questions Olenev's statement thus: 'I have no precise survival data on males but some of my culture tubes have contained females and males, and others males only, yet there was no obvious difference in length of survival'.

were killed by predators before the urge to activity possessed them. Of the remaining 50 %, some put in their last appearance later in the year of their emergence and others in the spring or early summer of the following year—the proportions depending on the earliness or lateness of the emergence. Obviously, the effective life of the adult stage is less than 1 year; and, from the turf-extraction experiments above, the actual life cannot be much more than 1 year. Incidentally, the two sets of experiments (Tables 6 and 7) are also somewhat in agreement on mortality rates.

By artificialities—among other things, the speeding up of development—the activity picture for Hethpool is distorted to some extent in Table 7. At this farm, there is normally only a spring activity, i.e. March–June in the case of adults. Judging from emergences in engorged nymphs collected from sheep in spring and kept in 100 % R.H. in an unheated room, it is easy to visualize the probable natural course of events (cp. lot 3, Table 7) as follows: Emerging in autumn (from a nymph engorged during the previous spring) the adult remains quiescent until the following spring when it becomes active; if it does not achieve a host then, it will be ineffective, i.e. die, without reproducing, before the next activity season.

Table 6. *Showing the percentage survival of experimental unfed adult ticks after different periods on the ground in hill grazings*

No. of experiments	No. of ticks laid down ♂♂	No. of ticks laid down ♀♀	No. of days on the ground	No. of live ticks recovered ♂♂	No. of live ticks recovered ♀♀	Percentage recovered ♂♂	Percentage recovered ♀♀
15	300	300	7– 88	177	222	59·0	74·0
12	218	256	104–192	80	136	36·7	53·1
3	57	70	217–272	29	38	50·9	54·3
2	40	40	358–368	0	0	0·0	0·0

In the course of the larger study of behaviour (Lees & Milne, in preparation), the writer made observations on 404 identifiable unfed adult individuals. These were deprived of normal hosts by large, ½ in. mesh, wire-net cages, but otherwise under natural conditions on the hill grazing at Hethpool. Starting when the ticks were laid down, the observations covered 22, 25 and 27 months, being made almost daily during 'activity' and at other times on fewer days evenly distributed over each month. As mentioned before, unfed ticks come for varying periods to the vegetation tips during the activity season. For each experimental tick, Table 7 shows the month of final appearance at the tips, i.e. the effective survival time, since a host can only be procured from the vantage point of the tips and oviposition (or development) is impossible without a blood meal. The data for males and females were practically identical and so have been treated together. About 50 % (206 in 404) of the experimental ticks were never seen after being set free on the grazing. Presumably these died 'naturally' or

Fig. 1 illustrates mortality among unfed ticks with and without vegetation shelter in spring and summer 1947. Unfed adults were collected by blanketing the pastures. The ticks blanketed in any one week were divided into two equal lots. Lot 1, confined in a Monel metal gauze envelope was buried in the upper mat; lot 2, in an open-meshed bolting silk envelope, was suspended at about the average height of the taller vegetation tips immediately above lot 1. Several similar experiments were carried out, and Fig. 1 gives the results of two typical experiments with females. The picture was the same with males, except that survival time was less both in and out of shelter. The shorter survival time of the males may have been the result of copulation (see before), since males and females were in the same envelope. At the vegetation tips, ticks had died of desiccation; in the mat, they had not died from this cause. Clearly, survival was much longer in the shelter of the mat, although,

even there, death would occur before next spring at the latest. For ticks confined at the vegetation tips, survivals ranged from 11 to 46 days, mean 32·7 (Exp. I), and 11–66 days, mean 36·0 (Exp. II), but the observation intervals were too wide for great precision. During the period of experiment daytime humidities were above normal on the whole.

Table 7. *Effective survival time for unfed adult ticks deprived of normal hosts but otherwise living in natural ground conditions.*

All adults were derived from nymphs engorged in spring 1945, incubated at 23° C., and stored in cool darkness at 100 % R.H. until set out in the field during 1945. Lot 1 consisted of four units set out on 7 May; lot 2, one unit, 27 July; and lot 3, four units, 12 October. Excepting one unit in lot 1 containing twenty males only, each unit comprised twenty-four male and twenty-four unfed female ticks, each individual identifiable by a system of single or double cellulose paint dots in four colours. The table shows the last calendar month in which each tick was actively seeking a host, i.e. was seen at the vegetation tips.

Month	No. of days of observation	Nos. of experimental ticks seen active for the last time in each month: Lot 1	Lot 2	Lot 3
1945: May (7)	10	0	—	—
June	23	0	—	—
July	22	12	0	—
August	24	17	1	—
September	22	5	3	—
October	23	1	1	1
November	26	1	0	0
December	11	0	0	0
1946: January	8	0	0	0
February	9	0	0	0
March	15	7	0	13
April	26	7	5	24
May	27	7	7	28
June	23	10	10	33
July	17	0	1	9
August	14	0	0	1
September	8	0	1	0
October	9	0	1	0
November	7	0	0	0
December	2	0	0	0
1947: January	2	0	0	0
February (blizzard)	—	—	—	—
March	5	0	0	0
April	23	0	0	0
May	26	0	0	0
June	25	0	0	0
July	17	0	0	0
Total ticks seen	—	67	30	109
Total ticks never seen	—	97	18	83
Totals	424	164	48	192

3. ENGORGED FEMALES IN DIFFERENT TYPES OF VEGETATION LAYER

According to MacLeod (1932): 'When the gorged and fertilized female drops from the host to the ground, she seeks shelter and, after a period, proceeds to lay eggs. The eggs are laid at the roots of grass, and among vegetable debris.' In Russia, Shcheglova (1939) observes: 'In the experiments of N. I. Alfeev (1935), who found the places in which

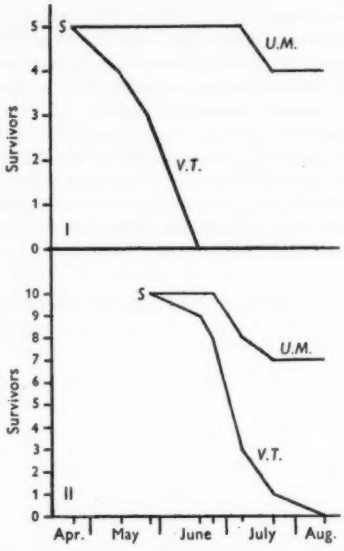

Fig. 1. Mortality among unfed female ticks in the shelter of the upper mat (U.M.) and with no shelter, i.e. at the vegetation tips (V.T.) immediately above, in 1947. Equal numbers of ticks laid down at the start (S) in each case. I, experiment with unfed females collected off the pastures by blanketing during the week ending 19 April 1947; II, similar experiment, 22 May 1947.

I. ricinus lays its eggs, thirty-eight out of ninety-eight experimental [engorged] females dug themselves into the ground to various depths (0·25–5·40 cm.). The rest of the females were found on the surface under lumps of earth or chips, or deep in vegetable cover.'

(i) *Methods*

Engorged females were collected from the floors of pens soon after they fell from sheep. They were generally dropped singly at the experimental points immediately, but a few were stored for some days at 100 % R.H. in a cool semi-dark room. These ticks could be seen easily because of their size and they

were observed *in situ* over long periods by simply inserting a thin-wire peg-label in the ground near each and moving the label when necessary.

(ii) *Behaviour (including lateral movements), vertical distribution, and survival*

(a) *In rough bent grass (deep vegetation layer)*

On the typical rough grass pasture of Hethpool, fourteen engorged females were dropped separately at different points from a height of 18 in. between 7 May and 19 June 1945. Observations were made on 33 days, spread over 7 May to 3 August, occasionally twice in the same day—morning and afternoon. During the observation period, six of the ticks were killed by predators; three went missing—probably carried off by predators; one died without violence, but not from desiccation; and the remaining four oviposited and died naturally afterwards. The ten which did not oviposit were laid down between 7 May and 11 June inclusive. Their survival times varied from 2 to 21 days, average 10.

bent at Hethpool—the 8 in. of growing grass stems on top, then the 1½ in. of comparatively loosely intertwined upper mat, and, nethermost, the 1 in. of densely tangled lower mat, with the stems of the growing vegetation passing through and toughening the mat. Resistance to penetration obviously increases with depth. Among the nine ticks of 1945 (including the four laying eggs) living long enough for the observation to be acceptable, downward progression ceased in from 2 or 3 to 18 or 19 days, mean 7–9. In three of the four completing oviposition, downward progression ceased after 2–3, 4–5, and 5–6 days respectively. The fourth of these ticks had got ¼ in. into the upper mat by the end of the first day after laying down and did not move again until between the seventeenth and nineteenth days when it was found to have gained a further ¼ in. downwards; thereafter it stopped finally. Among these nine ticks the limits of penetration were as follows: one got as far down as to be lying on the top of the lower mat, but the remaining eight got no further than ¼–¾ in. down from the top of the upper

Table 8. *Lateral (horizontal) progression in sixty-seven engorged female ticks dropped between 16 April and 6 June 1947 at Hethpool*

	Days after dropping when lateral movement ceased	Total linear lateral distance covered* (inches)	Extreme radial distance from arrival point on ground	Total different directions of progression*
Range	0–32	0–11·5	0–11·5	0–5
Mean per tick	3·1	2·9	2·2	1·4

* As calculated from position at observation times; the actuals are greater.

The four completing oviposition were laid down between 6 and 19 June inclusive. Their survival times were 45–57 days, mean 52. The data, therefore, suggest that females engorging later in the season were more successful than those engorging earlier. This was confirmed in 1947 when 10 in 18, or 55·5 %, of ticks dropped between 16 April and 23 May failed to start or complete oviposition, and only 1 in 8, or 12·5 %, of those dropped from 27 May to 6 June oviposited (cp. also Table 10). The duration of preoviposition and oviposition periods in nature is, of course, variable. On a rough average from forty-seven females engorged in April or May 1947, each period lasts about 1 month.

On arrival on the ground, the engorged female is usually supported on the criss-cross of growing grass stems and rushes fairly high up in the vegetation layer. It immediately begins to seek a way downwards, clumsily and slowly. Its efforts appear grotesquely feeble because of the enormous disproportion of legs to swollen body; yet the tick manages to bore down through the dense labyrinth of vegetation, dragging itself out of sight. Section 2, i, *b* already describes the vegetation layers in the rough mat. This was confirmed by additional observations on sixty-seven ticks laid down between 16 April and 6 June 1947; all of these engorged females got down into the upper mat.

Movement horizontally (laterally), accompanying the downward progression is confined to the first few days in the upper parts of the vegetation. It is random and comparatively small. Some ticks scarcely change at all their positions in the horizontal plane. Among the nine ticks mentioned above, the greatest lateral change from the dropping point was 2 in. The 1947 observations, however, showed that although the mean change is about 2 in., the range may be 0–11·5 in. (Table 8).

Observations on ten engorged females in *Nardus* at Lordenshaws also confirmed the above findings. These observations covered the period 4 May–15 June and no more than 1–3 hr. elapsed between the dropping of the ticks off sheep in the pens and their laying down. There was the usual loss from predators which lessened the scope of the experiments. The behaviour of one tick is interesting: it dropped in the centre of a small rosette of living *Nardus*, too dense to allow the passsage of an

engorged female. To reach the upper mat, this tick required to climb up about 3 in. over the 'lip' of the rosette-cup, and then drop into vegetation which could be penetrated. The tick first quested round and after 15 min. was vainly trying to force its way down through the rosette bottom. After a further 15 min. it was 1½ in. up the inside of the cup, head upwards but motionless. It did not move during a further 30 min. and unfortunately the writer had to leave it then. Next day it was found on the surface of the upper mat outside the rosette. Another of the ticks, deliberately placed in the cup, similarly had left it within a day. A third, having landed by chance in another looser rosette, did not leave it but was apparently content to be head down in a close-fitting tube of living stems, its hinder end seen clearly from above.

(*b*) *In fine short sward intermingled with clumps of rough bent grass*

During the same period at Lordenshaws, observations were made on eight engorged females placed from time to time on short fescue grass at a point 3 in. distant from a large clump of *Nardus* (*N*). (*N* = nearest; the next nearest clump was about 12 in. distant). The edges of clump *N* rose abruptly about 9 in. above the lawn-like fescue sward. The first tick moved parallel to the edge of clump *N*, then made a loop, first towards and finally away from the clump. At the end of 30 min. it had got about 1 in. from its original position although it had travelled about 3 in., and was now about ½ in. farther away from clump *N*. Obviously its movements were quite random. Next day it was found at the edge of clump *N*, but was still in the fescues. Two days afterwards it was still at the same spot, but had got right down on the earth surface, the grass being about 1 in. long and not dense. There it stayed, hidden by slightly overhanging *Nardus* stems but not in mat, until it was eaten a few days later. The second tick disappeared between laying down and the first subsequent observation. The third, fourth and fifth were found to have got an inch or two into the side of clump *N*, and, moreover, into the upper mat of the clump. The sixth, seventh and eighth disappeared in the same way as the second. That is, out of eight ticks laid on short grass providing little or no shelter, the four survivors had blundered to the edge of the nearest rough *Nardus* clump, three of them actually getting into the upper mat of the clump.

(*c*) *In fine short sward with no intermingled rougher vegetation*

It remains only to describe what happens when engorged females are dropped on a wide expanse of uniform short fescue-type sward. This work was done in 1942 at Hethpool. The ground chosen, on top of a large knoll, had grass 1½–2 in. long, with a tough basal matrix (of stems, roots and a little decaying vegetable matter) ½–1 in. thick on a shallow dry sandy soil. The grass is kept short and uniform, like a densely turfed lawn, by the incessant grazing of sheep. This was clearly shown by the fencing of a portion: in a relatively short time the grass became luxuriant and more than 6 in. high. The appearance of the sward in its natural grazed state suggested that engorged female ticks would find extreme difficulty in penetrating the matrix, and indeed would find it difficult even to descend through the closely set growing grass to the top of the matrix.

A cage, 5 yd. in diameter and 1 yd. high, of ½ in. mesh wire-net was erected in order to keep birds out, as the exposed ticks were very conspicuous. At different points in this cage, six engorged females, newly fallen from sheep, were dropped on 9 June; and six more on 26 June. Observation for 1–2 hr. after dropping showed that all the ticks made attempts to 'get down', moving aimlessly over the surface of the grass, trying here, trying there. The weather continued very hot and sunny. Naturally, the ticks made more horizontal linear movement here than on a penetrable sward, but the maximum radial distance achieved from the point of laying down was in the end only 3 in., excluding the missing tick (see below). Of the first six ticks, one was dead, shrunken and black, on the second day, and one was missing. The remaining four were still alive but slightly shrunken on the seventh day; and still more shrunken on the thirteenth when movement stopped, only one tick having moved (½ in.) since the second day. On the seventeenth day three were alive but in a bad way, i.e. shrunken and darkening in colour; the fourth was dead, black and desiccated. Sometime between the seventeenth and forty-ninth days the last three died. Their intact, shrunken, blackened bodies were found with no eggs on the forty-ninth day. Apart from the missing tick, none had 'got down' out of sight. The missing tick must have 'got down' somewhere nearby, however, because unfed larvae were found near its position the following spring.

After laying down the second lot of six ticks the writer could not, unfortunately, follow them up, but close search on the thirty-second day revealed three intact, black and desiccated bodies.

(*d*) *Conclusions*

When dropped at random on the ground, as they would, falling naturally from a host, newly engorged female ticks immediately seek to move downwards in the vegetation. The strength of this urge may be gauged by the behaviour of the ticks in the *Nardus* rosettes.

On very rough bent pasture, there may be a little random movement laterally but the total radial distance achieved is negligible (about 2 in. from arrival point), and the amount of movement is obviously restricted to the minimum necessary for finding a way down through the labyrinth of vegetation. It is confined to the period when the tick is in the uppermost parts of the vegetation. With the increasing density and toughness of the layers, lateral penetration would become progressively more difficult as the tick descended. All the ticks make their way into the mat. Downward progression stops short of the lower mat and generally is no deeper than the topmost half of the upper mat. Thus engorged females are distributed at a somewhat higher level than unfed females (cp. §2, i, *b* for the same pasture). Perhaps the engorged female is unable to penetrate any farther because of its bulk. In the mat the engorged tick, if it escapes predators, oviposits, completing the task about 2 months after arrival on the ground.

On fine sward intermingled with clumps of rough bent, ticks falling on the fine sward may blunder into the shelter (mat) of a rough bent clump, providing it is not too far away (perhaps no more than 3 in.). Failing that, they descend as low as possible in the fine sward.

On a large expanse of fine short sward with no intermingled rough vegetation, the tick, finding no downward path to shelter on arrival, turns this way and that, aimlessly, with the result that most of its progress in the horizontal plane is cancelled out. Thus, although the linear horizontal movement is greatest on this type of sward, the maximum radial distance achieved from the arrival point is again negligible, being no more than about 3 in. The great majority of engorged female ticks fail to get under cover and die of desiccation before they are ready to oviposit.

Summing up. On dropping to the ground, the first urge of the engorged female is to get down (vertically) under cover. In a deep vegetation layer it gets down into the upper mat with very little lateral deflexion, and there oviposits successfully. If cover is insufficient under its arrival point, however, it makes random lateral sallies, seeking a way down; but it cannot attain cover which is more than about 3 in. laterally from the arrival point. On a short sward providing no accessible cover, it dies of desiccation before ovipositing in a normal summer.

(iii) *Predators*

Some ticks fall victim to self-deticking hosts (Milne, 1948). No doubt others are licked up from grass tips and swallowed by grazing cattle and sheep. The tick also has true predators preying on it while in the ground microhabitat. Predators possibly include birds, small mammals, reptiles and arthropods. The following observations were made on the rough pastures of Hethpool and Lordenshaws.

(*a*) *Preliminary observations, with special reference to birds*

Table 9 shows the fate of eighteen engorged female ticks dropped at Lordenshaws in 1945. Observations were made on 5, 7, 11, 14, 18, 22, 23, 25 and 30 May; 1, 4, 6, 8, 11, 13 and 15 June. Each tick was seen alive on these days unless otherwise stated. All ticks 'found killed' had met their end in the same characteristic fashion, viz. they had been torn open, most of the 'shell' (body wall) usually remaining with the contents generally wholly, but occasionally only partly, cleaned out. Sometimes there was a long gaping rent in the shell; sometimes a largish portion of the shell, roughly rectangular or circular, had been excised; sometimes the shell was cut into two parts, occasionally one of the parts was missing. The margins of the severed cuticle generally had a fairly regular saw edge. As to the 'missing' individuals: the conspicuousness of the engorged female, the careful labelling and diligent searching, all justify the idea that they had been devoured entirely.

Table 9. *Fate of engorged female ticks under natural conditions on rough sheep pasture on Lordenshaws in* 1945

Tick	Date of dropping	Date when missing or dead
1	4 May	Found killed on 7 May
2	4 „	„ 11 „
3	7 „	„ 11 „
4	11 „	„ 14 „
5	11 „	„ 14 „
6	11 „	Missing on 14 May
7	18 „	Found killed on 22 „
8	18 „	„ 22 „
9	18 „	„ 22 „
10	18 „	„ 22 „
11	18 „	Missing on 22 May
12	18 „	„ 25 „
13	25 „	„ 30 „
14	25 „	„ 30 „
15	25 „	„ 30 „
16	25 „	„ 30 „
17	25 „	„ 1 June
18	25 „	Still alive on 15 June when observation ended

Observations for Table 9 ended (15 June) before egg laying would have commenced (cp. Table 10). Only one tick survived up to 15 June, and it had been on the ground 21 days. Eight ticks disappeared; and nine were found killed as described. That is, the great majority of engorged females perished before they could lay eggs. They died after periods ranging from not more than 3–7 days (av. 4·5) on the ground, or,

because of gaps in observation times, not less than 1–4 days (av. 2·8). Sometimes, on picking up a dead tick, a small spider or beetle would scuttle from inside the shell; these may have been scavengers, however, and the writer did not manage to capture any then.

At Hethpool, after similar experience on typical hill pasture, the writer collected a fair sample of the tussock arthropod fauna: spiders, mites, centipedes, millipedes, ground beetles, etc. These were kept for a week or more with engorged female ticks in bottles, but did not prey upon the ticks. The tentative inference, therefore, was that predators were chiefly confined to vertebrates.

eleven ticks dropped, only four survived to complete oviposition and these were dropped in June. None of the other seven even started to oviposit: one died a 'natural' death, i.e. was not killed by a predator; another disappeared after 7–9 days; the remaining five were all killed in 2–20 or 1–18 days after dropping, averages 13 or 10·4 days. The longer survival of missing and killed, as compared with Lordenshaws, could be due to differences of time and place in addition to the exclusion of birds.

Table 11 shows that about the same proportion (roughly half) of the ticks were killed characteristically on both farms before they could begin ovipositing. Birds were not excluded on Lordenshaws.

Table 10. *Fate of engorged female ticks under a bird-proof cage on rough pasture at Hethpool in* 1945. *Small mammals, reptiles and arthropoda not excluded by the cage*

Tick	Dropping date	Date when missing or dead	Oviposition
19	22 May	Missing on 31 May	None
20	22 „	Found killed on 31 May	None
21	22 „	Found killed on 11 June	None
22	22 „	Found killed on 11 June	None
23	22 „	Found dead and intact on 16 June	None
24	6 June	Found killed on 8 June	None
25	6 „	Missing on 3 August	Egg cluster complete and intact; oviposition began 19–25 June
26	11 „	Found killed on 25 June	None
27	11 „	Found dead, spent and intact on 3 August	Egg cluster complete and intact; oviposition began 26–28 June
28	12 „	Found dead, spent and intact on 3 August	Egg cluster complete and intact; oviposition began 19–25 June
29	19 „	Missing on 3 August	Egg cluster roughly average size at completion; oviposition began 9–23 July

A cylindrical wire-net cage, ½ in. mesh, 2 ft. 6 in. diameter, and 3 ft. high, like an inverted tin minus lid, was now used to exclude birds. The bottom rim was pressed down on the thick springy grass, but mice and shrews, etc., could easily enter through subsurface passages or tunnels within the lower depths of the vegetation or under the soil surface. Within the cage, five engorged females were dropped, well spaced, on 22 May. A new tick was then added for each casualty as occasion arose. In all, eleven females were used up to 3 August. Observations were made on 28, 29, 31 May; 1, 5, 6, 8, 9, 11, 14, 16, 18, 19, 25, 26, 28 June; 2, 4, 5, 9, 23, 28 July; and 3 August. This covered the egg-laying period of the females used. When oviposition was completed, further observation was unnecessary, since females then die naturally in any case, though some are attacked. The devouring of spent females is of no consequence to a tick population. Table 10 shows the data, each tick being seen alive on the days stated above except as indicated.

All those 'found killed' had met their end in the characteristic fashion already described. Of the

Table 11. *Fate of engorged female ticks dropped on rough pasture on Lordenshaws and Hethpool in* 1945

Ticks classed in percentages. Data from Tables 9 and 10

Condition	18 ticks on Lordenshaws (%)	11 ticks on Hethpool (%)
Killed characteristically	50·0	45·4
Missing	44·4	9·1
Survived	5·6	36·4
Died 'naturally'	0·0	9·1

Therefore the smaller proportion of 'missing' and the correspondingly larger proportion of 'surviving' ticks on Hethpool as compared with Lordenshaws suggest that birds must account for some of the 'missing' under natural conditions. The bulky, awkward, engorged female is not able to penetrate easily or quickly into the mat, and therefore is rather conspicuous for a time. Birds tend to swallow insects whole. Poultry greedily eat the ticks dropped in sheep pens.

(b) The common shrew and arthropods

The findings of 1945 suggested that (1) preoviposition mortality among engorged females was high in nature; (2) birds probably account for some by swallowing them whole; (3) arthropods are probably not very important as predators; which leaves (4) small mammals and reptiles to account for most mutilated and some missing ticks. The striking similarity of the condition of all ticks found killed suggested the work of one species or group of related species. Reptiles are scarce. The culprits might be 'mice'. The 1946 work was directed towards this question. The writer experimented with canister traps sunk in 'mice' runs on the rough grazings at Hethpool and Lordenshaws.

The trap is the type used against voles in forest plantations. It is simply a large cylindrical tin with an inward and downward projecting lip. The trap is sunk in a 'mouse' run so that the top is flush with the floor of the run. Both voles and shrews fall in and cannot clamber out because of the lip. These animals do not appear to live more than a day entrapped, and probably for less. Mice proper are not usually found out upon the open pasture where these experiments were made.

In very wet weather the trap may fill with water to a depth of 0–2 in. Fortunately this occurred rarely so that very little of our work had to be discarded on this account.

At Lordenshaws, two canister traps were put down on 10 May. Trap 1 contained ten engorged female ticks, trap 2 contained seven, all the ticks lying on the bottom. There was no change in the ticks on 13 and 20 May; but on 24 May, trap 1 contained three dead common shrews (*Sorex araneus*), one large ground beetle, a few spiders, the typical empty shell remains of three ticks and no trace of the other seven ticks; trap 2 contained spiders and ground beetles of the same and different kinds, but all seven ticks were intact and alive.

The ground beetle in trap 1 was kept for further experiment (see below) and the traps cleaned out. Trap 1 was immediately 'set' again with five fresh female ticks (two more being added on 7 June); and trap 2 was similarly reset with four females. The traps were visited every 3 or 4 days thereafter. At each visit, ground beetles, spiders, millipedes, etc., were removed; none of the ticks had been attacked by these. On 12 June, trap 1 again had its quota of ground beetles, etc., but the ticks were still untouched and alive; at the same time, trap 2 contained one dead shrew as well as the usual arthropods, but only the typical shell remains of all four ticks.

Thus the ticks survived the presence of the tussock arthropod fauna, but when shrews got into the traps the ticks either disappeared completely, i.e. were devoured, or were rent and cleaned out in the characteristic fashion described for 1945.

The large ground beetle taken from trap 1 on 24 May had been immediately confined in a small bottle with three half-engorged and two fully engorged female ticks. For two days the beetle paid no attention to the ticks, but by the third day it had killed the three half-engorged females. The shells were cleaned out but the rents were at least one-third to one-half shorter than those typical for 1945 or for the trap data above. Moreover, the margins of the rents were not regularly serrated but fairly even like an irregularly undulating scissors-cut. Hence the ground beetle is not the main culprit. Indeed it is very doubtful if ground beetles attack live animals in nature. They are chiefly carrion feeders. In the traps they quickly fell upon dead shrews and other arthropods. Confinement in the restricted area of a small bottle in the warm interior of a motor car, with no choice of food, is a poor test of the beetle's attitude to live ticks. There was one interesting feature: the beetle attacked only the half-engorged ticks. The latter are flattish and the beetle had cut them on the narrow edges of the body. Perhaps its fully opened mandibles could just compass these; the rounded fully engorged females with their tough cuticles should yield no scissors-grip. The beetle was now transferred to the more natural conditions of a herbarium containing several fully engorged female ticks on a turf cut from the pasture. The ticks were still alive and well even after 6 weeks.

Simultaneously, at Hethpool, the trap studies were extended with variants, the chief being that the female ticks were suspended, by threads fixed to their appendages, about 1½ in. from the bottom of the trap. Here the ticks were out of reach of beetles, etc., but could be taken by 'mice'. Four traps were used, shifted occasionally to new sites, and examined daily, except Sundays, from 9 May to 2 July. There were one to three, usually two or three, female ticks hanging in each trap.

On four separate occasions a single short-tailed vole (*Microtus agrestis*) had occupied one of the traps by itself but the voles never attacked the ticks.

Table 12 shows that on nineteen occasions a total of thirty-one common shrews were caught. These had available to them forty ticks.

Twenty-four ticks (60%) were killed by the shrews. Some of the killed ticks were dragged down to the floor of the trap, their appendages being left hanging in the threads; others were rent or severed, and cleaned out as they hung, the whole or part of the shell being left suspended. Details were as follows. Seven had been devoured, shell and all, thus proving finally that some of the 'missing' in nature are due to shrews. Of the remaining seventeen: twelve had been killed and cleaned out in the

characteristic fashion of 1945; while five had been killed by a single incision (bite) but not cleaned out. The serrated edge to the severed cuticle arises, of course, from the shrew's teeth pattern.

Sixteen ticks (40%) were unharmed in the presence of shrews. One had been pulled down but not damaged. The remaining fifteen were left hanging alive and intact in their threads. Of these, two had perhaps been suspended too high for the ticks. Although the herbarium, $10 \times 6\frac{1}{2}$ in., was abnormally highly populated with arthropod captures, only two females out of eight were killed from 21 May to 2 July. The others laid their eggs. One of the killed females had a small antero-ventral incision in its body wall; the other had a piece of body wall (about one-quarter) excised leaving more irregular indentations than is the case with shrew attack.

Table 12. *Showing number of shrews caught, and number of ticks in the trap and their fate on nineteen occasions.*

In the trap		Fate of ticks			
Shrews	Ticks	Completely devoured	Cuticle rent; innards eaten	Killed by a bite; innards not eaten	Unharmed
1	1	1	—	—	—
1	3	—	—	1	2
1	3	—	—	1	2
3	2	—	1	1	—
1	3	3	—	—	—
1	2	—	1	—	1
3	2	—	2	—	—
1	2	1	1	—	—
1	2	—	—	—	2*
1	2	—	2	—	—
2	2	—	2	—	—
2	2	—	—	—	2†
2	2	—	—	1	1
1	2	1	—	—	1
2	2	1	1	—	—
2	2	—	—	—	2†
2	2	—	—	—	2
2	2	—	1	1	—
2	2	—	1	—	1‡
Totals 31	40	7	12	5	16

* Shrew only half-grown, see text.
† On these occasions the shrews had been fighting.
‡ This tick pulled down by the shrews but unharmed.

captive shrew which was only half-grown; four were probably ignored because the shrews were fighting; seven were untouched while other ticks in the trap at the same time were killed; only once did the shrews ignore ticks for no apparent reason, two being untouched in a trap with two shrews which had not been fighting.

As pointed out in the last paragraph, one tick was pulled down unharmed. When the writer visited the trap, twelve large ground beetles were busy devouring the dead shrews; other arthropds such as spiders were also present; but no animal was attacking the tick. As noted earlier, in the course of the Hethpool work, many ground beetles, spiders, centipedes, etc., i.e. a good sample of the tussock fauna, were taken in the traps. These were always transferred to a herbarium (other than the one already mentioned) containing engorged female

(c) *The major predators*

The 1946 observations had established the shrew as a major predator of engorged females, and further suggested the insignificance of arthropods. In 1947, the writer laid down nine experiments, A–K, of five engorged females each, on the rough grazing at Hethpool. In Exps. A, D and G no cage was used, i.e. the ground was open to all natural predators; in B, E and H, birds were excluded as in 1946; in C, F and K, mammals, birds and reptiles were excluded by a cage of $\frac{1}{4}$ in. mesh wire cloth, sunk into the ground not less than 8 in. Ticks were laid down simultaneously in each group, beginning 16 April. From then until the end of July (106 days) observations were made on 66 days evenly distributed over the period. Table 13 shows a summary of the results. This confirms the 1945 and 1946 work

as follows: owing to predators, preoviposition mortality is very high in nature; shrews and, perhaps to a lesser extent, birds are the major predators.

4. VERTICAL ENVIRONMENTAL GRADIENTS IN A DEEP VEGETATION LAYER

(i) *Methods*

A previous paper (Milne, 1945) deals fully with air (shade) temperature, relative humidity and evaporativity at the vegetation tips throughout spring, summer and autumn. The present section shows the relation between temperatures and between humidities at tips and at different lower levels in a deep vegetation layer. With some knowledge of general climate in addition, the microclimatic gradients within the vegetation throughout the year can be inferred.

For study, the writer chose a heavily infested area of deep rough bent which includes the loci of experiments noted in §§2, i, *b* and 3, ii, *a*. Though uniform in a general way, closer inspection revealed a not inconsiderable variation in vegetation depth and density from point to point. The main variation was quite regular in that it consisted, about 50/50, of higher 'clumps' with intervening lower 'hollows' more or less evenly interspersed (see Milne, 1944). Readings were taken in the clumps. One factor influencing this choice was that gradients would be more easily detected in the deeper parts of the vegetation. Clumps were from 9 to 12 in. high.

In an area of about 5 acres, two random clumps were taken anew on each of about 45 days, evenly spread over the period 25 March to 29 May 1940; and similarly over 16 April to 30 June 1941. In each clump the following were recorded simultaneously: temperature and relative humidity at the tips and within the topmost $\frac{1}{2}$ in. of the mat; and temperature within the top inch of soil. For tips and mat readings the writer used small thermometers and watch-type hair-hygrometers, the latter frequently standardized against a wet-and-dry bulb thermometer and compared with one another; and for soil temperatures the requisite thermometer with a brass guard. At the vegetation tips, the instruments were hung on a small louvered frame, mounted on a pointed rod for thrusting into the ground; the frame was placed so as to cut off direct sunlight and had a short roof to keep off rain. In the mat, the instruments were inserted carefully into cavities made by the fingers, the surrounding vegetation being gathered round and over them immediately. Thermometers were given 10–15 min. to come into equilibrium, but hygrometers required 20, 30 or more minutes. Hair-hygrometers are not very sensitive to differences over the range 95–100 % R.H.

The evaporativity study was confined to a single typical clump. One Piche evaporimeter was suspended so that its effective surface was about the average height of the vegetation tips; another, immediately below, inside a gauze cage of diameter just greater than the evaporating paper, had its effective surface about mid-mat depth, i.e. towards the bottom of the upper mat. The evaporimeters were read several times a week and total weekly evaporation noted.

A thermohygrograph in a screen at tips height provided a continuous record of relative humidity and shade temperature.

Table 13. *Fate of engorged female ticks dropped at random on the rough grazing at Hethpool in* 1947

A, D and G, open to all predators; B, E and H, birds excluded; C, F and K, all vertebrates excluded, i.e. open to arthropods only

	A	D	G	B	E	H	C	F	K	A+D+G	B+E+H	C+F+K
Missing before starting oviposition	3	3	3	3	1	1	0	1	0	9	5	1
Killed before starting oviposition	1	0	0	0	0	1	1	0	0	1	1	1
Killed before completing oviposition	0	0	0	0	0	1	0	0	1	0	1	1
Completed oviposition	1	2	2	2	4	2	4	4	4	5	8	12

(ii) *Temperature gradients*

Temperature gradients in vegetation and underlying soil layers are complex. Besides ordinary radiation, conduction and convection effects, there are major air movements such as winds to take into account. The latter are constantly bringing masses of colder or warmer air over the ground, and the temperature of the macroclimatic atmosphere, or rather its heat capacity, has a very potent day to day and seasonal effect on that of the ground microclimate. This effect obviously decreases with depth since the temperature a few feet beneath the soil surface is unaffected by day-and-night changes at the surface. Within the levels relevant to the present paper, the decrease of macroclimatic effect with depth may be illustrated by simultaneous readings at vegetation tips, within the topmost $\frac{1}{2}$ in. of the upper mat and the topmost 1 in. of the soil. The following is a typical section of the data, covering

46 observation days between 16 April and 30 June 1941:

	Tips	Upper mat	Top soil
Range (° F.)	42–82	41–78	46–70
Mean (° F.)	56·7	54·1	53·6

Obviously, in going from spring into summer, the general level of temperature decreases with depth; in going from summer into winter the opposite will be true. Again, the range of fluctuation also decreases with depth. This may be still better illustrated, using the same section of data, by averaging the 'day to day' (i.e. observation days only) temperature differences at the three levels:

'Day to day' change (° F.)	Tips	Upper mat	Top soil
Range	0–21	0–13	0–8
Mean	6·60	5·00	2·29

The blanketing effect of the vegetation also produces a time lag between macroclimatic and microclimatic changes. One example will illustrate this. It covers 3 days, on two of which there are available the temperatures at the three different levels for a particular time of day, and on all of which, the screen temperatures from the continuously recording thermohygrograph:

Date	Mean day (24 hr.) temperatures	Temperatures* at Tips	Upper mat	Top soil
20 April	45	—	—	—
21 ,,	49	65	58	54
22 ,,	41	44	45	48

* 3 p.m. 21 April; 11 a.m. 22 April.

The macroclimatic temperature fell considerably from 21 to 22 April but the corresponding falls at tips, upper mat and top soil were as 21:13:6, i.e. the rate of fall decreased with depth. Again, the rising temperature from 20 to 21 April and the falling from 21 to 22 April produced opposite gradients vertically due to the time lag.

Summarizing. Microclimatic temperatures follow the macroclimatic. Temperatures at vegetation tips are always very near the macroclimatic. According to rate and range of macroclimatic fluctuation and depending on the moment of reading, one may find on proceeding vertically downwards in the vegetation that either the temperature rises or falls, or rises and then falls, or falls and then rises. On the average, however, the deeper in the vegetation the lower the temperature in summer, and the higher in winter. The longer a macroclimatic temperature prevails, the nearer will microclimatic temperatures approach those of the macroclimate. At all times, temperature fluctuation range decreases with depth in the vegetation.

The continuously recording thermohygrograph showed that shade temperatures at the vegetation tips vary from below freezing-point to a little over 80° F. (27° C.) throughout the year (Milne, 1945). These are extremes, of course, and for most of the year temperatures are more or less far from being so low or so high. From what has been said above, the tick experiences a shorter temperature range within the mat.

(iii) *Humidity gradients*

The following facts have already been noted (Milne, 1945). At the vegetation tips, relative humidity fluctuates over a widish range throughout the year; for instance, at Hethpool, from 100 down to 40% R.H. In 1942 at Hethpool, the monthly average of minimum daily percentage relative humidity in the screen from April to October varied from 59 to 74%, the grand average (31 March to 2 November) being 66%. At the tips, 'there is an almost continuous diurnal fluctuation in percentage relative humidity related to diurnal fluctuation in air temperature. Except during rain, mist, fog, etc., humidity falls to its lowest point during the heat of the day and rises to its highest during the cool of the night.' Moreover, tips humidity invariably rises to 95–100% R.H. every night, no matter how low it has previously fallen during the day. Typically, during April–June at Hethpool, tips relative humidity is greater than 92% for 5–6 hr. and less than 80% for 9–10 hr. of the 24 hr. day; over April–October, roughly 8 hr. in both cases (Table 1, op. cit.).

Now the vegetation encloses a layer of air which is relatively still and partially insulated from the macroclimatic atmosphere. For this and other reasons (including transpiration of living plants and water-holding capacity of dead vegetable matter and soil), microclimatic humidity must necessarily differ often from macroclimatic. This could be illustrated by tabulating the many simultaneous readings at tips and in mat showing no gradient in rain or mist or at night, and a steep gradient during the day in dry periods; but such variations are to be expected. Table 14 better illustrates the general daytime humidity difference with depth by showing the distribution of recording-occasions in percentage relative humidity categories over the tick activity season. The data are for 1941 (those for 1940 give a similar picture); they cover 44 days evenly spread over the period 16 April to 30 June. Somewhere between 10 a.m. and 4 p.m., two clumps were studied each day and there was little variation between them. Accordingly, the data have been treated together so that there are eighty-eight recording occasions in all. In the top of the upper mat, 90% of trials showed humidities of over 80% R.H., while at the tips only 24% of trials did so over the season; and the mean humidity was

higher and the fluctuation range narrower in the top of the upper mat. Table 14, therefore, clearly suggests that there is generally a daytime humidity gradient in the vegetation layer: humidity increasing and its fluctuation decreasing with depth. Lees (1948) shows that the gradient is steepest at the point where the longer stems penetrate the tangled growth of shorter grass, i.e. just above the mat.

Table 14. *Distribution of recording occasions in percentage relative humidity categories for readings of humidity at vegetation tips* (V.T.) *and within the topmost half an inch of the upper mat* (U.M.).

Data for 44 days between 16 April and 30 June 1941, in a deep layer of rough bent at Hethpool (see text).

R.H. (%)	Recording-occasions V.T.	U.M.
45– 49	2	0
50– 54	4	0
55– 59	11	0
60– 64	18	0
65– 69	8	1
70– 74	15	3
75– 79	9	4
80– 84	11	3
85– 89	2	9
90– 94	4	28
95–100	4	40
Ranges in relative humidity	48–100	68–100
Mean	70·4	91·7

In the top of the upper mat, humidity varied little in 2½ months, the averages for the four consecutive eleven-(observation)-day periods being 89·8, 91·4, 92·6 and 92·9 % R.H. Note that the readings were taken not at ½ in. depth but within the top ½ in.; in practice the writer aimed at having the perforations in the hygrometer casing about ¼ in. deep in the mat. Now to a tick at that depth, the humidity may well be somewhat higher than the readings indicated because (*a*) opening up the vegetation to insert the comparatively bulky instrument, (*b*) the difficulty of resettling the vegetation around it, and (*c*) the longish wait for it to come into equilibrium, necessitating several readings—all these circumstances would tend to give a lower humidity than that actually existing in the tick niches of undisturbed vegetation. Hence, it is reasonable to suppose that, at a depth of ½ in. or not much more in the mat, the relative humidity will always be over 90 and nearer 100 % in dense deep vegetation layers of the type now under study. Indeed, this was confirmed by inspection, the general body of the upper mat being damp to the touch and the lower damper and often wet even in the height of an ordinary summer. The mat is sponge-like, retaining water between the replenishing intermittent rains and nightly dews, and is aided in this by the blanket of growing vegetation hindering the drying action of sun and wind. So strong is this water-holding property that in the course of a prolonged drought the writer once found the upper parts of the living vegetation parched, the underlying soil dry and crumbly, but the intervening mat fairly damp on a well-drained slope of the Hethpool rough grazing.

Table 15. *Total cubic centimetres of evaporation per week (ending on dates shown) at vegetation tips and within mat for the year* 1944 *on rough grass at Hethpool*

Date	Within mat	At tips
1 May	0·20	19·4
8 ,,	0·60	23·6
15 ,,	0·00	30·5
23 ,,	0·08	12·7
29 ,,	0·40	30·8
5 June	0·10	13·8
12 ,,	0·00	16·7
19 ,,	0·00	25·4
26 ,,	0·00	20·8
3 July	0·00	7·3
10 ,,	0·00	12·8
17 ,,	0·00	9·5
24 ,,	0·00	9·2
31 ,,	0·00	4·7
7 August	0·05	10·5
14 ,,	0·00	12·1
21 ,,	0·00	14·7
28 ,,	0·00	12·0
4 September	0·15	4·2
11 ,,	0·10	6·0
18 ,,	0·20	10·4
25 ,,	0·00	4·3
2 October	0·00	13·3
9 ,,	0·00	3·9
16 ,,	0·00	8·6
24 ,,	0·00	4·2
30 ,,	0·00	6·2
Total	1·88	347·6

The evaporativity readings taken *towards the bottom* of the upper mat confirm the idea that relative humidity well within the upper mat must always be verging on 100 % in the deep rough bent in summer. From 1 May to 30 October, Table 15 shows practically no evaporation (total 1·88 c.c.) within the mat compared with heavy evaporation at tips (347·6 c.c.). The rather higher evaporation rate in the mat for May was coupled not only with the obvious dryness but also with the new opening-up of the living vegetation and mat by the gauze cage. Later, the vegetation grew close around but the cage would always permit more air circulation than normal; and there was some disturbance of the upper vegetation every week when the evaporimeter was withdrawn from the cage in order to change the paper. In short, what little evaporation there was may have been an exaggeration from the tick's viewpoint.

5. RELEVANT PHYSIOLOGY

This section gives a brief outline of what seem to be the most important physiological facts in relation to the microhabitat environment.

(i) *Water relations*

Lees (1946) finds *in vitro* as follows:

For the unfed tick 'a state of equilibrium is attained at a relative humidity of about 92%; at lower relative humidities the tick loses water by evaporation, while at higher humidities it takes up water....Near the point of equilibrium the loss or gain of water over a wide range of temperature is determined by the relative humidity....The uptake of water from humid air occurs when the tick is in a desiccated condition but ceases as the normal water content is restored.' Within reason, repeated fluctuations between low and normal water content do not have any apparent ill-effect on the tick.

The engorged tick loses water more rapidly than the unfed, and loses it slowly even at 95% R.H. 'The engorged tick usually lacks the ability to take up water from humid air'; if previously desiccated, it fails (as might be expected from the preceding sentence) to take up water from an atmospheric humidity as high as 95% R.H. 'Both unfed and engorged ticks possess the ability to prevent or limit temporarily the entry of water in contact with the cuticle.' In prolonged contact with water, ticks swell up. In time complete immersion kills (asphyxiates?). If placed in saturated air after a few days exposure to water, the water content falls but in some cases 'the loss of fluid may continue long after the normal balance has been restored and in extreme cases the tick may dry up. This takes place in spite of the fact that the ticks were usually active and apparently unharmed when removed from contact with water.'

(ii) *Behaviour*

In the laboratory, Lees (1948) has made a very thorough study of all the likely factors concerned in behaviour. He finds as follows:

In the engorged stage, ticks are strongly photonegative and thigmotactic; after desiccation they are intensely active but soon come to rest in moist air.

In the unfed stage: 'The humidity behaviour is greatly influenced by the physiological state. When their water balance is normal, unfed ticks avoid higher humidities....The avoiding response (taxis) disappears after desiccation and is replaced by a kinesis: the tick is then intensely active in dry air but soon comes to rest in moist air. On remaining in damp air the water balance is restored by water uptake through the cuticle. The first response then reappears. The operation of the kinesis does not appear to depend on sensory stimulation.'

Various stimuli, such as vibration, light, temperature and smell, are concerned in the tick's grasping and attaching to a host.

(iii) *Survival*

In the introduction to his paper, Lees (1946) summarizes MacLeod's work thus: 'The various stages of *Ixodes ricinus* are known to display little resistance to desiccation. This is true of the egg (MacLeod, 1935*b*) and of the unfed nymph and larva (MacLeod, 1935*a*). Moreover, the optimal humidity for larval and nymphal development over a wide range of temperatures is saturated air, development failing to proceed below a relative humidity of about 80% (MacLeod, 1934).'

With males, unfed females and engorged nymphs, 'previously maintained in saturated air in the culture tubes', Lees (1946) made comprehensive observations (*in vitro*) on rate of water loss and on survival in different humidities at 25° C. Results for unfed females may be summarized thus:

R.H. (%)	Rate of water loss per day (% of original body weight)	Survival (days)
0	Up to 50	1
30	Up to 20	2–3
50	Up to 10	3–5
70	Up to 5	4–8
90	Negligible for longish period	7–14 to 56–84
95	Negligible for long period	Usually 84 or more

Here the experimental ticks were of various ages from 1 to 4 months. In a private communication concerning further work on the subject, Lees stated: 'The ageing of the unfed tick has a marked effect on the resistance to desiccation. The newly emerged tick reaches equilibrium at about 88% R.H., but as the age increases so the equilibrium humidity also rises and, correspondingly, the resistance to desiccation diminishes. The very old tick is thus only in equilibrium in saturated air.' Lees has since (10 December 1946) kindly supplied the following unpublished data showing average survival in days at two widely differing relative humidities, using groups of ten unfed female ticks of known age:

Age (months)	Humidity at 25° C. 95% R.H.	50% R.H.
1	238·0 days	5·9 days
4	167·8 „	4·0 „
8	104·7 „	3·8 „
12	12·6 „	2·8 „

Because 25° C. represents only the extreme upper end of the temperature range met by the tick in nature, Lees then undertook a series of survival observations *in vitro* with newly emerged females, all of the same age, at constant temperatures from

5 to 25° C. and constant humidities from 0 to 95% R.H. (Table 16). These data confirm his findings for resistance in ticks about 3–4 months old (cp. the results for 50% and 95% R.H. at 25° C.) and the fact that survival time not only increases directly as the relative humidity but is enormously increased at relative humidities over 80%. The data also show that survival time increases as the temperature descends.

Table 16. *Survival of unfed female ticks at different temperatures and humidities*

The ticks used were from a batch which emerged over a 3-week period. At the beginning of the experiment the average age was 12 weeks.

Conditions (% R.H.)	Survival (days)					Mean survival (days)
	25° C.					
0	2	3	3	3	4	3·0
50	2	3	5	5	6	4·2
70	4	7	8	10	10	7·8
85	39	65	109	109	113	87·0
95	65	200	214	236	271	197·2
	19° C.					
0	4	4	5	5	7	5·0
50	3	4	4	6	11	5·6
70	8	13	15	20	20	15·2
85	5	39	73	101	116	66·8
95	88	281	292	376	403	288·0
	11° C.					
0	2	4	6	8	8	5·6
50	3	3	7	8	15	7·2
70	4	7	9	24	38	16·4
85	5	17	65	81	153	64·2
95	130	221	234	257	682	304·8
	5° C.					
0	7	9	9	10	14	9·8
50	7	9	10	19	21	13·2
70	22	23	29	38	43	31·0
85	15	22	79	112	213	88·2
95	38	48	206	564	720	315·2

In addition, Lees (same personal communication) has made the interesting finding that 'in some years a high proportion of unfed male and female ticks blanketed from the pasture in spring are highly susceptible to desiccation. This might be taken to indicate that the active ticks are fairly aged on the average.* On the other hand, work now in progress is throwing emphasis on the pronounced effect of activity on the depletion of the tick's reserves; such depletion probably reduces both the resistance to desiccation and the survival time. Susceptible ticks from the blanket may in some cases be comparatively young individuals which are near the end of a period of intense activity.'

* Between 6 and 10 months will have passed since they emerged as adults (see § 2, iii).

6. DISCUSSION AND CONCLUSIONS

The tick passes by far the major part of its life span on the ground (§ 1). In a deep vegetation layer such as presented by rough hill or moorland pasture, the ideal habitat (Milne, 1944), the female tick's ground life is as follows: moulting from the engorged nymph in autumn, it remains quiescent in the upper mat until, if it survives, activity sets in during March to June (Milne, 1945). Then the unfed female climbs periodically to the tip of the tallest piece of vegetation (stem, blade, frond, etc.) in its immediate vicinity where it settles down to wait (Lees & Milne, in preparation). While waiting there, it will clutch eagerly at a passing host, and, rationalistically, this is the best vantage point for so doing. At a lower level, other stems would almost always intervene between tick and host. The chances of a host coming exactly within reach must often be small. Yet the unfed tick spends only a minor part of the activity season at the tips, commonly five periods averaging about 4–5 days each if no host is forthcoming (Lees & Milne, *ibid*). Between periods of activity and afterwards, it is quiescent in the upper mat and dies before the next spring activity season, probably by the end of summer (§ 2, iii). On the other hand, if it achieves a host between March and June, the engorged female, after falling to the ground, immediately descends and spends the remainder of its life within the upper mat; owing to the activity of predators, preoviposition mortality is high; oviposition is complete about 2 months after engorgement, the female dying soon after (§ 3, ii, iii). This picture is true of the male also, except that it gets on the host to find a female, not to feed, and dies after mating. From superficial observation it appears that the same behaviour sequence applies to nymphs and larvae, except that development to the next stage is substituted for reproduction, after engorgement. Environmental conditions and physiology throw light on this microhabitat economy (§§ 4 and 5).

Apart from inactivation by low temperature and from the host-responses of the unfed stage, the main physical factors governing the adult tick appear to be humidity and light for the engorged stage and humidity alone for the unfed stage (§ 5). The responses to these factors are either immobility or vertical progression (§§ 2–5).

In its immediate seeking to move downwards after falling to the ground, the engorged tick is no doubt responding to its strong photonegativism, since humidity would scarcely have had time to come into play. In coming to rest in the upper mat, perhaps light conditions, thigmotropism and increasing impenetrability all have some influence at first, but humidity will later be the ruling factor. The engorged tick requires conditions verging very nearly on saturation in order to preserve its water

balance—it loses water slowly even at 95% R.H. (§5, i). Usually, in summer, constant humidities of this order occur no higher than in the upper mat (§4, iii). Perhaps the deeper 'burrowing' of some females many days after first coming to rest (§3, ii, *a*) and the behaviour in the *Nardus* rosette-cup (§3, ii, *b*) are expressions of the kinesis noted in the laboratory (§5, ii), the original position ultimately proving too dry.

Placing his behaviour facts (§5, ii) alongside the existence of a steep humidity gradient in the natural microhabitat (§4, iii), Lees (1948) reasonably suggests humidity response as the factor governing the hungry tick's alternation between tips and mat, thus: 'Certain reactions guide the tick...to the vegetation tips....The tendency to remain near the tips is assisted at first by the humidity response, for ticks walking on the stem lattice avoid the high humidity near the roots. After an unsuccessful period of waiting, the tick becomes desiccated [this being hastened by activity, see §5, iii] and the kinesis comes into play: walking downwards the tick comes to rest at the roots, takes up water from the damp atmosphere, and is then prepared for a further period of activity at the tips.' With April–June macroatmospheric conditions at Hethpool typically below 90% R.H. (equilibrium level in youngish ticks, see §5, i) for about 18 or 19 hr. of the day (Milne, 1945) it is logical to think that water gain would not balance water loss at the vegetation tips. The tick will certainly be able to make up lost water in the saturated atmosphere of the upper mat (cp. §§4 and 5).

The data in Fig. 1 suggest an average survival time of about 4 or 5 weeks for normal unfed spring females compelled to remain continuously at the vegetation tips. Survival time in ticks so hindered is no argument against humidity conditions being the prime reason for the periodic shorter stays at the tips. No doubt the kinesis sets in long before desiccation is so advanced as to cause very much discomfort, let alone be dangerous to life. Further, a survival of 4 or 5 weeks must be somewhat unusual in nature since in 1947 the hours per day at above 90% R.H. were higher than average, viz. about 12 or 13 for each of the months April–July as opposed to the 5 or 6 hr. noted above.

Undoubtedly humidity is one of the most important factors in the tick's natural life. It is only unimportant during the short time the tick is attached to a host, because then the necessary water is derived from the imbibed blood (Lees, 1946). The process of finding and leaving a host brings the humidity factor into play. §5 shows that the engorged stage is even more susceptible to desiccation than the unfed stage. The engorged stage has to suffer the driest time of year—summer. Its point of arrival back on the ground is random, depending on the wanderings of the host. Of its own efforts it can achieve little or no change of position on the horizontal plane, even when that is necessary (§3); its chief response is to 'burrow' down vertically as far as it can. Vegetation depth and structure vary over a pasture; therefore sometimes the amount of cover immediately under the tick's point of arrival may not ultimately maintain a sufficiently high humidity for survival. Adding all these facts together, one may suggest that the weakest link in the tick's life chain is presented by the engorged stage. For, if the engorged stage develops successfully, the resultant unfed stage, living during less droughty seasons, will not suffer from niche humidity-unsuitability.

Summing up. The unfed tick's domestic economy presents itself as an unconscious conflict between the need to achieve a host and the need to conserve water. The engorged tick, on the other hand, need only conserve its water, but is more susceptible to desiccation; as it occurs in summer, the driest time, the engorged stage is the more vulnerable from the humidity viewpoint. The behaviour and physiology of the tick (§§2, 3 and 5) suggest that it will be most efficient in a vegetation layer providing ready access to relative humidities constantly at, or very near, 100%. In the deep layer of a rough hill or moorland pasture, the tick can easily make its way down to the upper mat where such humidity conditions exist, and, indeed, spends most of its unfed and engorged life there (§§2–4). Significantly, other things being equal (including host-potential), the tick is most successful in such rough pasture, as judged by population density (Milne, 1944).

7. SUMMARY

Since it is concerned with filling in certain details of a picture, long since sketched in outline, the new work in this paper permits only a somewhat disjointed summary.

From a study of *Ixodes ricinus* L. in nature, the main new facts emerging are:

(I) *Unfed adults in a deep vegetation layer* such as presented by rough hill or moorland pasture dominated by bents, heather or bracken. (i) Practically all *inactive* unfed ticks are in the underlying mat, and nearly all of these in the upper portion of the mat. This distribution does not change, summer or winter. (ii) When the tick is active its progression is practically confined to the vertical, i.e. between its niche in the mat and the vegetation tips immediately above. Random undirected movements due to certain circumstances may achieve a 0–8 in. (average 2 in.) change of position in the horizontal plane; but there is apparently no ruling urge to move in the horizontal plane. (iii) The unfed tick comes to the vegetation tips to await a host for only a limited

time during the 3-month activity season. Commonly this amounts to about five periods of 4–5 days each if no host is forthcoming. Between the periods of activity it returns to the upper mat. If it stayed constantly at the tips its survival time would be much shorter than normal. (iv) From nymphs engorged in spring, adults emerge in autumn but remain inactive for a considerable period. About half of them may perish within the first 100 days after emergence. Survivors become active during the following March–June. Effective life ends when the tick, if still unfed, finally stops coming to the vegetation tips, the only position from which a host can normally be achieved. After emergence, the effective life of the unfed adult deprived of a host is less than one year; and the actual life is not much more than one year at best. Thus adults not finding a host in spring will be ineffective, i.e. die without reproducing before the next activity season.

(II) *Engorged females.* After arrival back on the ground, the ruling urge for the engorged female is to proceed vertically downwards into the cover of the vegetation. Lateral (horizontal) progression is random and confined to the minimum necessary for finding a way down through the labyrinth of stems. (i) On a short dense sward providing no accessible cover, engorged females die of desiccation before ovipositing in a normal summer. (ii) On a short sward with 'islets' of long bents, they apparently cannot attain cover which is more than about 3 in. from their arrival point on the ground; at that distance some do manage to blunder into cover. (iii) In a deep vegetation layer such as rough bent grass, they get down under cover in the upper mat and, if they escape predators, complete oviposition after about 2 months. The proportion of ticks achieving oviposition is larger among those engorging later in the spring-activity season. (iv) Owing to the activity of predators preoviposition mortality is high. The common shrew and, perhaps to a lesser extent, birds are the major predators.

(III) *Humidity gradients in a deep vegetation layer.* (i) At the vegetation tips: relative humidity varies from 100 down to 40%, returning each night to 100%. For more than half, usually three-quarters, of the 24 hr. day it is less than 90% during the spring-activity season; for just under half the day it is less than 80%. (ii) In the upper mat: at the level reachable by ticks, relative humidity is constantly at, or very near, 100% throughout the year.

The above findings are woven up with known physiological (summary given) and ecological facts in an attempt to give a more complete picture and understanding of tick economy within the microhabitat (see text).

The behaviour and water relations of the tick suggest that it will be most efficient in a vegetation layer providing ready access to a constant relative humidity of 100%. In the deep layer of a rough hill or moorland pasture, the tick can easily make its way down into the upper mat where such humidity conditions exist, and, indeed, spends most of its unfed and engorged life there. Significantly, other things being equal, the tick is most successful in such rough pasture, as judged by population density.

The writer is very grateful to Mr T. P. McNicol, Experimental Officer, for much help in laboratory and field; to Mr J. Allan Campbell, B.A., M.R.C.V.S., Edinburgh University, for introducing him to the flotation-separation method in §2, i; to Dr J. MacLeod of the Cooper Technical Bureau for criticism of the MS.; to Dr V. B. Wigglesworth, F.R.S., Director, Agricultural Research Council Unit of Insect Physiology, for his interest and for comments on the MS.; and especially warmly to Dr A. D. Lees, also of the Unit, for incubating material, for doing some of the laboratory experiments in §5, for unselfish giving of his unpublished work, and for much useful discussion and constructive criticism.

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APPENDIX

Details of male and unfed female tick material used in Tables 1–3. All ticks came from north-east Northumberland except those in turves 12–15 at Hethpool, which came from south-west Cumberland. Males and unfed females were developed from engorged nymphs (collected from sheep) in incubators at 20–23° C., 'hatching' of a batch being usually complete about 2 months after setting up in the incubator. After metamorphosis, and until put down on the grazings, the adults were stored in a cool room in semi-darkness at 100 % R.H.

Engorged nymphs collected during	Resulting adults laid down on grazing at	Turf containing adults lifted at	Turf no.	Location
April–May 1944	10 July 1944	1 August 1944	1	
,,	,,	7 August 1944	2	
,,	,,	14 August 1944	3	
,,	4 August 1944	21 August 1944	4	
,,	,,	28 August 1944	5	
,,	,,	26 September 1944	6	
,,	,,	31 October 1944	7	
,,	,,	4 December 1944	8	
,,	,,	8 January 1945	9	
,,	,,	12 February 1945	10	Hethpool rough grass
,,	,,	26 April 1945	11	
September 1944	12 February 1945	7 June 1945	12	
,,	2 March 1945	16 July 1945	13	
,,	12 June 1945	27 August 1945	14	
,,	,,	24 September 1945	15	
April–May 1947	24 June 1947	30 June 1947	16	
,,	,,	7 July 1947	17	
,,	,,	12 July 1947	18	
,,	,,	19 July 1947	19	
May 1944	29 September 1944	19 December 1944	1	
,,	,,	23 January 1945	2	
,,	,,	19 February 1945	3	
April 1945	15 June 1945	15 November 1945	4	Lordenshaws bracken
,,	,,	14 March 1946	5	
,,	,,	18 June 1946	6	
May 1944	29 September 1944	3 November 1944	1	
,,	,,	12 December 1944	2	
,,	,,	16 January 1945	3	
,,	,,	27 February 1945	4	Lordenshaws heather
April 1945	15 June 1945	8 November 1945	5	
,,	,,	18 January 1946	6	
,,	,,	18 June 1946	7	

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THE ECOLOGY OF THE SHEEP TICK, *IXODES RICINUS* L.

SPATIAL DISTRIBUTION

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1. INTRODUCTION

'Many observers record that the tick (*Ixodes ricinus* L.) is most commonly found in swampy, undrained areas, or where a coarse vegetation of shrubs, bracken or heather is present. Olenev (1927), from a study of the moisture requirements of the tick, was able to show that this choice of habitat was due to the high degree of humidity necessary for its survival and development. His conclusions have been confirmed by the writer....' So said MacLeod (1936) introducing the subject of 'habitat requirements' in 'an analysis of the ecological complexes controlling distribution...'. By distribution MacLeod meant geographical presence or absence of the tick. His 1936 view of distribution in Britain was subsequently repeated by himself in various forms (1938*a*, *b*, 1939, 1941). His work and view may be summarized as follows. First he indicated the absence of the tick on lowland pastures (1932, 1936). Then, in the laboratory (1934*a*, 1935 *a*, *b*) he showed (as stated (1939)) that 'under summer conditions of temperature the unfed tick requires for survival an aqueous vapour tension equivalent to over 80 % saturation, and the gorged tick 85 % saturation or over for development, and 80 % or over for oviposition'. From this he claimed that the tick 'remains confined to the hill pastures' (1936) because only there does it meet those 'definite conditions of soil and vegetation' (1938*a*) providing the requisite humidity in summer. The latter conditions were defined thus (1938*a*): 'The soil must be of such a nature that it remains damp even during the summer months, e.g. marsh or peat, or soil covered with moss. Over the soil there must be a fairly deep cover of vegetation, e.g. heather or bracken, or old bent- and moor-grass, growing year after year until a mat of withered herbage is formed.' Thus MacLeod believed microclimatic humidity in summer to be the 'all-important' factor limiting distribution. That belief was based mainly on reasoned conjecture for, in contrast to his considerable laboratory work, his field study of environmental factors (1936, p. 304, §*a*) and indeed of the tick's ecology (1932) was rather scant.

Lees and this writer (see references later) have now enlarged MacLeod's work both in laboratory and field. As the last in the present series on the field ecology, the following paper attempts a modification and extension of MacLeod's theory of distribution. There are two ways of looking at spatial distribution: (i) mere presence or absence of the species (as MacLeod, above) or (ii) density of individuals. The former is only a limited aspect of the latter since 'absence' is zero density. In the present paper, spatial distribution connotes density over the land surface at a given time. Spatial distribution must not be confused with chronological distribution, the latter being population density in time at a given place. The tick does fluctuate in numbers at any place (and in unison at different places over very

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large areas) with some semblance of a three-year cycle (unpublished); but it does so moderately. It does not multiply enormously and then suddenly decrease to innocuous numbers. It is more or less of a pest all the time (cp. Milne, 1945*c*). Hence this writer has concentrated on the spatial rather than on the time aspect of distribution. With species which are pests only towards the peak of their cycle, both aspects are economically important, and in some cases the time aspect is more important. With the tick, it is more useful to know (*a*) why density varies from place to place, than (*b*) what factors check unlimited increase in the most favourable place.

2. BRIEF SURVEY OF FACTS AND IDEAS ON LIMITING FACTORS

I. ricinus has two basic requirements from its habitat: (*a*) suitable ground conditions for survival when awaiting a host, and for development after achieving a host; (*b*) a host for food and mating purposes. One would, therefore, expect spatial distribution to be a function of both requirements. Except for showing that an appreciable tick population is maintained by the wild fauna in the absence of sheep (1934*b*), MacLeod paid little attention to the host factor, on the reasonable grounds that the tick is never absent through lack of hosts to maintain it.

(i) *Ground cover*

(*a*) *Correlations in nature between amount of cover and tick distribution*

The writer investigated geographical and local distribution in relation to geology, soil and vegetation in northern England (Milne, 1944, 1946). The main findings were: (1) The tick is practically confined to rough hill and moorland pastures; the latter, in sharp contrast to most pastures where the tick is absent or very scarce, have a dense relatively thick layer of vegetation. These observations, based on fuller data, confirmed those of MacLeod (see §1). (2) Depth of vegetational cover varies over a pasture; and when the tick is present on a pasture its density is positively correlated with depth of cover.

(*b*) *Experiments on effect of reducing cover*

The writer also proved experimentally (Milne, 1948*c*) that lessening the amount of cover (agricultural improvement of rough pasture) brings about a significant reduction in tick population. Clearly, amount of cover is a prime controlling factor in tick distribution.

(*c*) *Discussion of means whereby cover limits tick population*

The controlling effect of amount of ground cover may be due to associated humidity in summer, as MacLeod conjectured (see §1).

The tick's need of high humidities, for development and survival, has already been described at length (Olenev, and MacLeod, see §1; Lees, 1946; Milne, 1948*b*). Since the tick has to wait until a host comes within reach, and since the host-seeking periods of the individual are comparatively short (Milne, 1948*b*), it follows that duration of survival in the unfed stage must be of prime importance from the population viewpoint. Maximum survival is possible for the unfed tick only if it has access to relative humidities between 88 and 100%, the equilibrium level rising with age. Below its equilibrium level the tick desiccates and survival time falls away steeply as the humidity decreases (Lees, 1946; Milne, 1948*b*). The engorged tick is still more susceptible to desiccation, and loses water slowly even at 95% R.H. (Lees, 1946). For engorged ticks, MacLeod's work (1934, 1935*b*) indicates the following mortalities before moulting or ovipositing under constant conditions *in vitro*: (1) as humidity falls away from saturation, mortality begins to rise steeply somewhere between 100 and 95% R.H., in the case of larvae at least; (2) at relative humidities between 85 and 80%, practically all individuals of all stages die. Thus readily accessible niches (crannies) with humidity verging closely and constantly on saturation are a necessity for the tick; and, indeed, complete saturation is required for maximum efficiency from the population viewpoint.

Departures from the required niche humidity condition for efficiency should be correlated with depth of cover. Obviously the shallower the vegetation layer the greater the liability to some degree of desiccation in summer or abnormal droughts. This would be due not only to lesser overall depth and density of sheltering vegetation, but also to less underlying mat since the latter varies directly as the former. Mat has a sponge-like water-retaining property. A thinner mat has a less reserve of moisture.

Without cover, the tick (fed or unfed) dies of desiccation comparatively soon outdoors in a normal spring or summer; in *Nardus* about 9 in. deep with mat 2½ in. thick, it spends most of its life somewhere within the top 1½ in. of mat, at which level the relative humidity is constantly at, or very near, 100% (Milne, 1948*b*). Table 1 and §2, iii, will show that such vegetation conditions exist on one part (plot 3) but not on another (plot 5) of the same pasture on Hethpool farm. Plot 5, besides having much shallower vegetation and much less mat, also has a considerably lower tick density (cp. §2, i, *a*). Towards the end of a summer drought, sample inspection showed that, while the mat of plot 3 was expectedly damp, the basal parts of plot 5 were dryish to the touch. This strengthened the idea of a humidity cause for the positive correlation between amount of cover and tick population density.

Humidity comparison of plots was not pursued quantitatively, however, for compelling reasons. The engorged tick drops at random on the ground and it is restricted to a very small radius, about 2 in. on the average, round its point of arrival in the surface plane (Milne, 1948*b*). It will die too soon if it does not chance upon a suitable niche during its random movements within that radius. Obviously, then, the relevant circumstances in cover are the humidity classes and area-densities of the deepest niches attainable by the tick. (1) Among the infinite variations of composition, depth and structure within even a single superficially uniform plot, and (2) with no means of identifying a single ultimate niche in the absence of an occupant, nor (3) of recording its undisturbed humidity (available instruments being too bulky, see Milne, 1948*b*), clearly it would be futile at present to attempt precise plot-humidity comparisons relevant to tick population. In short, the data which would put the humidity theory beyond question are unprocurable.

There is yet some evidence, however, which may be adduced for the humidity theory. Improvement of rough pasture (overall lessening in vegetational depth and density and partial or complete destruction of underlying mat and mosses) is followed not only by a general reduction in tick population (see §2, *i*, *b*), but also by a relatively greater reduction in nymphs than in adults (Milne, 1948*c*). This strongly suggests a desiccation effect since smaller stages dry up more easily (MacLeod, 1935*a*; Lees in private communication) as might be expected from surface/volume considerations.

The importance of humidity, as associated with physical character of vegetation, in the success of other pasture-dwelling arthropods has also been suggested by Ford (1937) in his studies of fluctuations in natural populations of *Collembola* and *Acarina* in grass tussocks.

There seems, then, to be quite a strong case for the theory that cover effects control on ticks mainly through associated humidity in summer. At the same time, ticks should survive through an abnormally wet or damp summer in poor cover.

Strong as the case for humidity seems, however, insufficient weight may have been given to the temperature factor. MacLeod conjectured (1938*b*) that 'temperature may occasionally affect the species' (presumably in poor cover) and (1939) that ticks are protected from lethal extremes of temperature by suitable ground cover. In the field, as would be expected, the present writer did indeed find that temperature fluctuation range decreases (at both ends) with depth under ground vegetation cover (see Milne, 1948*b*), but no information is actually available as to the precise 'extremes of temperature' obtaining under any depth of cover. MacLeod's final word was (1941): 'the physical factor controlling distribution of the tick as a species has been shown to be moisture during summer, with, possibly, temperature during severe winters'.

Between 5 and 25° C. *in vitro*, survival time of ticks increases as the temperature decreases, whatever the relative humidity may be (see Milne, 1948*b*). This may be an effect of saturation deficiency. At all events, in summer, when microhabitat moisture is normally at its lowest, temperature is at least conditioning humidity unfavourably.

In winter, on the other hand, humidity is probably never a limiting factor because the ground then is normally saturated. Amount of cover will still be important, however, if temperatures approaching those of the macroclimate can be lethal. By bringing the temperature down from 16° C. in 3–8 days and up again in 2–6 days, MacLeod (1935*a*) showed that 'the tick in all its [unfed] stages can survive exposure for 4 days to a temperature of −8° C. It is killed by 1 day's exposure to a temperature of −15° C.' He concluded that 'the tick shows considerable powers of resistance to feezing'. That may be true, for his experimental ticks (habituated to laboratory temperatures?) were subjected to an abnormal range of fluctuation and had little time to develop cold-hardiness; but such an experiment provides a dubious basis for conjecture as to the tick's resistance in nature. Most ticks have to survive in the unfed stage not for a few days but right through winter (Milne, 1948*b*). Diurnal fluctuation of temperature between levels above and below freezing-point is often considerable. In cold periods there is not always a protecting blanket of snow. Without field trial, one cannot exclude the possibility that temperature conditions in poor cover might be lethal in a normal winter. This should certainly be investigated by any future worker.

It has been noted above how the engorged tick is more or less restricted to its point of arrival on the ground when it drops from the host. Its subsequent unfed stage is also similarly restricted to that point (Milne, 1948*b*). Clearly the lower the area-density of suitable niches the less would be the chances of the random-dropped tick surviving. One way, therefore, of explaining cover-effect would be to assume that density of niches physically suitable in any degree is positively correlated with average depth of cover. This seems a reasonable assumption when one thinks of the universal irregularity of vegetation depth and structure in any kind of pasture.

Apart from hosts, the most important biotic environmental factor on the ground should be the activity of predators. The common shrew and, perhaps to a less extent (because less numerous), birds are the chief predators of engorged female ticks (Milne, 1948*b*). One would imagine that the greater the amount of cover the less easily would ticks be found by shrews and birds. If so, then the

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activity of predators may also be an important associated circumstance in cover, for losses from predators are heavy in the best of cover. For example, in three experiments on pastures with ideal cover, 64 and 67% (1945) and 94% (1947) of engorged females were killed before ovipositing (Milne, 1948*b*). In the case of the 67% mortality, ticks had been distributed on the ground at about the average maximum density in nature. In the other cases their density was about twice average maximum but, in one case (64%), with ticks protected from bird attack.

(ii) *Host-potential*

In the tick, development from one stage to the next is impossible without a blood meal from a host. Moreover, adults being so sparsely distributed over the ground (cp. Milne, 1946, 1947*b*) and having an overmastering urge to move vertically and not horizontally in vegetation (Lees, 1948; Milne, 1948*b*), the sexes can meet only on the body of a host. Thus the tick depends on a host not only for its food, but also for the opportunity of mating. At certain times the unfed tick becomes active, i.e. climbs to the vegetation tips. There it awaits the passing of a host. The host-awaiting periods are comparatively few and short, e.g. the female individual has commonly about five periods, averaging 4–5 days each, spread over the 3 months of the general activity season of the population in spring (Milne, 1948*b*). Further, any unfed tick failing to achieve a host dies before the next activity season (Milne, 1948*b*). The chances of a host coming exactly within reach at the right time obviously depend on the abundance or scarcity of hosts. Hence, host-potential must have some influence on spatial distribution.

From work on host relationships (Milne, 1948*a*) the following facts emerge. Any available mammal or bird, wild or domestic, serves as a host. Compared with farm animals, the total wild fauna contributes very little to host-potential. The sheep flock feeds the vast majority of ticks of all stages and as much as 99% of females on a hill or moorland pasture in northern England. Therefore, if it is a question of relative host-potential on areas of properly stocked sheep-grazing land in this region, comparison of sheep densities alone is sufficient.

Certain data (Milne, 1947*b*) suggest that, on pastures with similar average depths of cover, total tick population is larger the denser the sheep stocking. The reason must be that fewer ticks perish for want of food and mating is more certain where, as a result of heavier stocking, the ground is more often and more thoroughly quartered by sheep.

The interplay of ground cover and host-potential factors is enlarged upon later (see §2, iii, below). Meanwhile, some additional facts and ideas about hosts and host-potential are worth noting here.

Although the sex ratio is unity,* a host will generally pick up unequal numbers of the sexes. Therefore, some females will return to the ground unfertilized and with no further opportunity of meeting a male. Moreover, on a large host like the sheep or cow the random searchings of a male for a female may sometimes be fruitless. Clearly, mating is not 100% even among those adults fortunate enough to achieve a host.

One sheep per acre is the highest stocking density met on hill farms. At that density, the flock on one of the smaller Hethpool pastures picked up between 15,000 and 45,000 female ticks over the entire spring season of 1944. 'Exhaustion' blanketing (Milne, 1946) showed that the more heavily infested *half* of this pasture actually had a female population of between 70,000 and 116,000 in that spring. These computations suggest the enormous numbers of ticks failing to achieve a host even under the best conditions (contrary to a conjecture in Milne, 1947*b*, p. 39).

Although necessary, the achievement of a host may be disadvantageous to a tick in the following ways: (1) A tick may be picked up on the tongue of a grazing animal and swallowed. (2) Depending upon its point of arrival, a tick may get 'lost' in the sheep fleece, and fleece conditions appear to be lethal in some measure (Milne, 1947*b*). The bulk of a sheep's infestation occurs on the bare or hairy areas of the body (Milne, 1945*c*), and in the fleece appreciable numbers of ticks are found which have died before becoming attached. (3) Once attached, a tick may be killed by its host. Thus birds and the smaller mammals account for a proportion of their infestation by self-deticking (Milne, 1948*a*), and perhaps by mutual deticking. This proportion must be fairly high in birds since attached ticks are found only on the head and upper neck, i.e. beyond beak reach. Again, from the irritation they set up, some ticks (especially engorged females) are killed by large hosts like sheep and cattle scratching and rubbing themselves against walls, trees, posts, etc. Moreover, repeated infestation may ultimately result in a host acquiring a skin reaction which prevents some attached ticks from engorging and may even cause their death. Such acquired immunity is often mentioned in foreign literature and the present writer has seen some evidence of it in this country. (4) Finally, having engorged successfully, a tick may be killed by its host shedding it upon ground unsuitable for survival.

* MacLeod (1932) gives the sex ratio as 9/5 in favour of females. In larger samples of engorged nymphs developed in incubators, the present writer and his colleagues found the ratio to be unity. Moreover, in blanket samples of unfed adults off pasture the ratio is also unity (see e.g. Milne, 1948*c*).

(iii) *Interplay of ground cover and host-potential factors*

Section 2, ii, indicates that host-potential is probably the chief limiting factor under equal conditions of ground suitability. Since better pastures (less cover) are more heavily stocked with sheep and cattle, the absence or scarcity of ticks on better pastures (§§ 1 and 2) suggests that amount of ground cover can override host-potential.

Host-potential is not uniform within a hill or moorland pasture. Typically, vegetation varies over such a pasture (Milne, 1944). Sheep have grazing preferences and definite resting places, i.e. host-potential is greater on some parts than on others. Sheep movements will, therefore, very largely determine the pattern in which engorged ticks fall on a pasture. In a previous paper (Milne, 1944), one set of data shows a positive correlation between vegetation depth and tick population density within a pasture, while a second set shows the same correlation between mat thickness and population. Since the deeper the vegetation the thicker the mat, it follows that both sets of data really show the same thing, namely, a positive correlation between tick density and depth of cover. Below is a reconsideration of the second set (summarized in Table 1), host-potential information being available for it alone.

Each plot in Table 1 was within a uniform area of the pasture containing it. At time of sampling, average vegetation depths ranged from about 2 in. for plot 5 to about 9 or 10 in. for plots 3 and 4, with plots 1 and 2 intermediate, plot 2 being deeper than plot 1. Plot 5 was fine fescue-type grass; plots 1 and 3, rough bent grass; plots 2 and 4, mingled rough bent grass and bracken.

The nymphal densities in Table 1 require some qualification. Another paper (Milne, 1943) stresses the need for similar evenness of vegetation surface in plots if the blanket-dragging method is used to estimate relative densities. An active tick comes very near or actually to the tip of the tallest stem, leaf or frond immediately above it on the ground (Milne, 1948*b*), but the blanket does not necessarily touch that tip in passing over. For instance, the blanket will ride clear of a tick on a *Nardus* stem if there are fully grown brackens round about. Generally, the thinner the vegetation layer the more even the surface, as indicated by the mat ranges in Table 1 (mat thickness being proportional to depth of vegetation). The more even the surface the more intimate the blanket application, i.e. the more tips sampled. Hence the density differences shown in Table 1 are possibly underestimated. Plot 1 may have yielded proportionately more of its active ticks to the blanket than plot 2; and plot 5 more than plots 3 or 4 (since blanketing was completed in early June, before the brackens had overtopped the intermingled bents, plots 3 and 4 had surfaces of similar evenness).

Observed differences in both nymphal populations and mat thicknesses (amounts of cover) are significant ($P = < 0{\cdot}05$) between plots 1 and 2 on the one pasture; and on the other between plots 3 and 5, and 4 and 5, but not between 3 and 4. In order to assess host-potential during the tick-activity season, the writer watched flock movements over several years. His observations were confirmed by the shepherds of the two pastures. The findings are as follows:

The pasture containing plots 1 and 2 is divisible into three distinct functional areas. There is a uniform bent grass area (A), actually an enclosure with several gates and 'creeps' open for sheep, containing plot 1. Adjacent to this is a broadly uniform bent grass and bracken area (B), containing plot 2. Above area B is the hilltop area on which the flock spends the night. The flock grazes area A in the middle of the day and area B earlier and later in the day. Time spent on areas A and B is about equal; but area B is between two and three times the extent of area A. Hence, since no part (plot) of a uniform area should attract sheep more than another, there must be more sheep on plot 1 than on plot 2 for the same length of time. As the sheep spend different times of day on the plots, however, can this be taken to mean that plot 1 actually has the higher host-potential? Both the picking-up and dropping of ticks are implied in host-potential. Since the data in Table 1 are measures of active population the relevant aspect of host-potential here is the dropping of engorged ticks on which the active population depends. There is the possibility that engorged ticks might have a lesser tendency to drop off in the middle of the day than at earlier and later hours. Some evidence discounting this idea has already been given (Milne,

Table 1. *Relative nymphal populations (by blanket dragging) and actual mat thicknesses (in inches) on five plots at Hethpool in* 1942

Plots 1 and 2 on one pasture; plots 3–5 on another. Each plot 6000 sq.yd. Data summarized from Milne (1944)

Plots ...	1	2	3	4	5
Nymphal densities	0·2	2·2	5·1	5·1	0·2
Mat thicknesses: Range	0·6–1·8	0·7–3·1	1·5–5·2	2·2–4·8	0·5–1·0
Mean	1·2	2·3	3·1	3·1	0·7

1946). Further, during 7 years of tick counting on sheep, the writer has never been aware of any obvious differential shedding of engorged ticks according to time of day in daylight. Hence, it seems justifiable to conclude from the available facts that host-potential on plot 1 is greater than on plot 2.

The pasture containing plots 3–5 has only two broad functional divisions. One is again a uniform, short and fine grassed hilltop area (*C*), where the flock spends the night. Plot 5 lies within this area. The other is the remaining area (*D*) of the pasture. This consists of stretches of bent grass or bent grass with intermingled bracken, whereon the flock grazes during the day. Plots 3 and 4 are adjacent about the middle of area *D*. The bracken on plot 4, not a dense infestation, is usually inconspicuous until late June. From mid- or late March until early June, when the bulk of tick activity generally occurs at Hethpool (Milne, 1945*a*, 1947*a*), the grazing values of plots 3 and 4 are alike—chiefly bent with intermingled fescue-type grasses. As might be expected, therefore, plots 3 and 4 receive equal attention from sheep, i.e. have equal host-potential during the period that matters; but area *D* is several times the extent of area *C*, although time spent on each area by the flock is about equal. Hence there must be more sheep on plot 5 than on plots 3 or 4 for the same length of time. The following additional considerations suggest that this may be justifiably interpreted as indicating a higher host-potential on plot 5. During the night, sheep lie down oftener and for longer periods than during the day. Probably, therefore, more ticks drop off during the night owing to increased friction aiding the process of disengagement. The extreme of cold during the night may possibly aid the disengagement also. Nuttall (1911) noted that replete *Boophilus* 'rattled like peas on the ground' when their host, a cow, was taken from its 'warmed experimental stall into the cool air of a Courtyard' in Cambridge.

The comparable facts may be summarized as in Table 2. If the measures of relative host-potential are valid,† as the writer believes, then clearly tick-population density is positively correlated only with depth of herbage (amount of cover) here. The negative correlation with host-potential indicates that, under the given conditions, ground cover has overridden host-potential to some extent. Since sheep tend to concentrate on the finer (less deep) herbage, cover may often be the master factor in tick distribution within a rough pasture; but cover effect will be conditioned by host-potential.

† A possible objection to the line of reasoning is that, where sheep stocking is denser, then, proportionately fewer active ticks might be left to the blanket; but counts on sheep temporarily confined to areas similar to *A* and *B* in every way on the other side of the hill showed differences of the same order as in blanketing.

3. A THEORY OF SPATIAL DISTRIBUTION

Broadly speaking, *Ixodes ricinus* is absent on lowland farms in Britain, being confined in varying population density to hill and moorland farms (MacLeod, 1932; Milne, 1944, 1946, 1947*b*). The following is a theory of this spatial distribution, which the facts as known at present appear to fit.

(i) *Distribution in general*

The greater bulk of the distribution seems to be adequately explained in terms of interplay between the two major environmental factors: (1) ground cover (i.e. amount of protection from adverse conditions of humidity and temperature, and from predators), and (2) host-potential (i.e. supply of hosts for feeding and mating purposes), with cover the master factor.

Table 2. *The relationship of host-potential and of vegetation layer depth to tick-population density on certain plots (see text and Table* 1)

Plots	Plots 3 and 4	Plots 3 (or 4) and 5	Plots 1 and 2
Host-potentials	Plots equal*	Plot 3 (or 4) < plot 5	Plot 2 < plot 1
Vegetation layer depths	Plots equal*	Plot 3 (or 4) > plot 5	Plot 2 > plot 1
Tick-population densities	Plots equal*	Plot 3 (or 4) > plot 5	Plot 2 > plot 1

* Approximately. < = less than; > = greater than.

Lowland pastures are generally shallow and structurally very open, whereas hill and moorland pastures are characterized by deep, dense layers of vegetation over much of their area. The tick is absent in the lowlands because cover is too poor for its survival in sufficient numbers to maintain permanent populations. MacLeod previously came to the same conclusion (§ 1).

On the hills and moorlands, tick population is, within limits, denser on a pasture: (1) the more universal the distribution of adequate cover, and (2) the higher the host-potential. (1) follows because: (*a*) a tick is more or less restricted to the random point at which it drops from its host (§ 2, i, *c*); and, (*b*), cover varies over any rough pasture (§ 2, iii). Any patch of poorer cover limits total population since upon that patch are dissipated some of the ticks from the better cover. In addition, the sheep's habit of lying overnight on poor cover must be a severe checking factor to multiplication because the sheep flock provides by far the major portion of the host-potential (§ 2, iii).

(2) follows because the more hosts the more thoroughly is the ground quartered and hence the fewer ticks starve or miss the opportunity of mating (§2, ii). The qualifying phrase 'within limits' was used at the beginning of this paragraph because obviously a point could be reached where increase of cover would be superfluous and increase of host-potential a hindrance. Thus, so far as the tick is concerned, ideal associated physical conditions would be reached at a certain minimum depth and density of cover; and since the chief hosts, wild as well as domestic, are herbivores, any increase of host-potential beyond a certain limit would tend to reduce cover by over-grazing.

(ii) *Tick 'spread'*

Although the above explanation is adequate for the greater bulk of spatial distribution, it is, nevertheless, incomplete. For examples, on the hills and moorlands there are some pastures, well-stocked and providing excellent cover, with no appreciable tick population, and other pastures with a lower population than they ought to have considering the amounts of cover and host-potential available. To meet these cases one must bring in the question of colonization of new territories by the tick, usually referred to as 'spread'. In the past this has been a very vexed and acrimonious question. Some have perhaps exaggerated the actualities and possibilities of spread (cp. Moore, 1938, 1939), while others have tended to minimize them (cp. MacLeod, 1938*b*; and Ministry of Agriculture opinion in Moore, 1938, 1939). Before going on to a new analysis of the question, it is worth reiterating two things: (1) on rough hill and moorland pastures, sheep are the chief farm animals with cattle a long way behind (Milne, 1945*c*); (2) the sheep flock is by far the main source of tick hosts, supporting the major part of a pasture population of young stages and, in most places, from 94 to 99% of females (Milne, 1948*a*). Among other things incomplete knowledge of (2) led the MacLeod school astray.

There are two aspects of 'spread': (*a*) marginal extension of existing foci of infestation, i.e. the 'spread by accretion' of MacLeod (1938*b*); and (*b*) creation of new foci at more or less distance from existing foci, i.e. with intervening tick-free land.

(*a*) *The reality of 'spread'*

Moore (1938, 1939) claimed that both kinds of spread were occurring to a somewhat alarming extent. Largely on theoretical grounds, MacLeod (1938*b*) took the view that the amount of spread occurring must be negligible on the whole, consisting mainly of marginal extension of existing foci into places where good pasture had deteriorated in favour of the tick. From farmers and shepherds in northern England, the present writer amassed a considerable body of evidence (Milne, 1940) indicating that the truth lay between Moore and MacLeod. A single example, from the College Valley area (Northumberland), will illustrate how, under suitable conditions, a new focus of infestation is created and how its margins slowly extend.

The substance of this particular example is mainly owing to a very intelligent shepherd, Edward Douglass (now deceased), who took a deep interest in the tick 'problem'. He worked as a hill shepherd all his days in the College Valley area, as his forebears had done. The last 27 years of his life were spent at the farm of Hethpool. From statements of his predecessors and from his own observations, he drew the following picture. Before 1860–70, the tick was unknown in College Valley and for miles around. About that time it was introduced to one pasture of Westnewton Tors, a farm contiguous with Hethpool. It came on a flock comprising sheep bought at May Day from the notorious infested areas of north Tyne in England and of south-west Scotland, 'where ticks had always been'. Since then, partly by transferring of flocks and partly by sheep-straying due to fence disrepair, the tick had gradually encroached upon pasture after pasture and farm after farm until today about 15–20 farms in that area are infested. Their rate of spread was slow. The original focus, Westnewton Tors, has been infested for about 80 years. Farms within a radius of roughly 2 or 3 miles have been infested for 30–60 years. Outside that, up to a radius of 5–6 miles from the centre, the infestations date from only 5 to 20 years back, and some farms are not infested. When Douglass first came to work on Hethpool, one of its pastures, the Ewe Hill, was still free of ticks. He unwittingly infested the Ewe Hill by transferring to it a flock from one of his 'ticky' pastures during high activity. Over the years he watched the tick population grow on the Ewe Hill until now it is the most heavily infested pasture on the farm. Details of the spread in College Valley area during the last 30–40 years were confirmed by other shepherds, active and retired.

The above example indicates the broad reality of spread. The mass of this kind of evidence shows that the tick colonizes most surely and its population comes into equilibrium soonest when a whole flock of sheep is transferred quickly from a heavily tick-infested to a suitable tick-free farm during peak activity (as about May Day). In such a case the flock brings up to one-fifth of the total ticks it would have fed on the pasture from which it came (see Milne, 1947*b* and §2, ii). Even so, the build-up process is, according to hill-men, comparatively slow. Ticks seemingly do not become very numerous until 15–20 years after introduction. This would explain some evidences of descending gradients in tick density towards the margins of the infested area centred on Hethpool and Westnewton Tors

(cp. Milne, 1944, table 2)—gradients not correlated with cover or host-potential.

As a body, hill shepherds are characteristically good observers of nature. Even if they were not so, the writer believes that their evidence of spread is largely dependable for the following reasons. Ticks are usually at the height of their activity on sheep during the 4 or 5 weeks of the lambing season (Milne, 1945*c*). A shepherd actually handles a good number of his ewes at lambing. He would be a very unobservant shepherd who did not notice the conspicuous engorged female (so unlike the ubiquitous ked, *Melophagus ovinus*) appearing on his sheep for the first time. Since MacLeod & Gordon (1932) incriminated *Ixodes ricinus* as the carrier of much of the disease trouble among hill sheep, the shepherd has become more and more 'tick conscious'. Nevertheless, critics are not wanting who scorn shepherds' evidence of spread with such suggestions as: no scientific observer has seen spread take place; some tick populations are disease free; the tick was there all the time only the shepherd did not take notice of it until mortality increased sharply after diseased sheep from elsewhere had infected the native tick population. How was one to satisfy such critics? Farmers know and fear the consequences to 'unacclimatized' sheep following upon the introduction of *I. ricinus*. Hence one had to discard the idea of direct experiment, especially because one knew that the tick cannot be eradicated from a hill pasture once it is established (Milne, 1945*c*, 1948*c*). Close observation of tick-free farms, however protracted, would be rather futile since the tick might never have the opportunity to colonize a single one of the limited number selected. If a consistent build-up of population could be demonstrated on a farm alleged to be recently infested, that might be adduced as evidence of colonization. This would take many years because, as in all wild species, population would have an independent cycle of numbers going on simultaneously (see § 1). There seemed to be no possibility of coming by the merest shred of direct evidence in a reasonable length of time when by sheer chance the following opportunity arose.

The writer had been investigating the possibility of physical environmental reasons for three farms on a line (Hethpool, Westnewton (not the Tors) and Kilham) showing a gradient in tick density (Milne, 1944). In so doing he made comparable tick counts on sheep at each farm in 1940. A fortunate (!) breakdown in a Kilham march fence and the perspicacity of the Kilham shepherd subsequently transformed these counts into the basis of the one bit of direct evidence of spread now in the writer's possession. On Kilham (Thompson's Walls farm) the pasture is, if anything, only a little less rough than on the heavily infested Hethpool, 3 or 4 miles distant along the Cheviots. In the week following peak activity in 1940, a random sample of ten Kilham sheep yielded no ticks while an identical Hethpool sample yielded a total of 659 females. Three weeks later the figures were 0 and 110. The Kilham shepherd maintained that in thirty-odd years on the farm he had never seen a single tick on his sheep. This was credible because, being a lifelong friend of the Hethpool shepherd (Douglass) whose sheep had suffered much from ticks, he was familiar with the tick's appearance and since the early thirties at any rate was constantly on the look-out for it. In 1942 he sent the writer word that he had seen 'an odd tick on an odd sheep' in his flock that spring for the very first time. A fence between his pasture and a fairly heavily infested pasture of a neighbouring farm (Elsdonburn) had fallen into disrepair during spring 1940 with the result that some infested sheep had been straying over for a time. Repeat examinations later by the writer showed total female ticks on ten sheep at peak activity to be as follows:

Year	Kilham	Hethpool
1945	7	214
1946	8	549

The writer was unable to make any observation in 1947 but the shepherd reported that ticks on Kilham 'were about the same as in 1946'. The tick had spread and established itself!

(*b*) *Ideas on the mechanism of 'spread', and their application to the problem of discontinuous spatial distribution*

Whether spread takes place must depend on, (1), the numbers of engorged ticks introduced, this being conditioned by, (2), the area-density of suitable niches (§ 2, i, *c*) and, (3), subsequent host-potential. Factor (2) determines the proportion of survivors from one season to the next; factor (3) the number of those survivors mating and/or feeding (§ 2, ii). In species unable to migrate actively (as *I. ricinus*, see § 2, i, *c*), whether a population maintains itself, grows, or dwindles, usually depends only on reproductive and death-rates. Reproduction in the tick is hampered by the sexes being unable to meet except on a host; and mating is not 100 % even among adults fortunate enough to achieve a host (§ 2, ii). In any set of circumstances there must be a critical level of adult population for the number of matings to be adequate for replacements in the face of normal checks. Above that level the population will grow until it comes into equilibrium with the environment; below, it will dwindle and disappear (also, of course, 'a coming into equilibrium'). The end-point of the latter case might be only one male and one female surviving from all the engorged ticks originally dropped on the pasture: the likelihood then would be that one host would pick up the sole male and another host the sole female, so that no

mating could take place. These ideas help to explain certain aspects of discontinuous distribution at the present time.

Referring to the present day, MacLeod (1938*b*) held that wild animals, because of their territorial systems, cannot be involved in spread of ticks to new areas far removed from established foci. With this the writer agrees. Man alone is responsible for such spread by moving comparatively large numbers of sheep, the major hosts, swiftly from place to place. Speed is essential since many or all of the ticks would drop off during a prolonged journey by droving or by transport.

MacLeod also believed that the wild fauna by itself would inevitably bring about marginal extension of existing foci if ground conditions were suitable. This requires some qualification. Although undeterred by sheep fencing, wild animals (red deer apart) must normally contribute little to marginal extension to-day. Their negligible collective host-potential and certain facts of distribution suggest this forcibly. Thus, occasionally, although separated only by a sheep fence from a long-established heavy infestation, a rough pasture may be found with no appreciable tick population. For example, Kilham (see above) is contiguous with Elsdonburn which has been fairly heavily infested for more than 30 years. In these cases, the few ticks brought on by wild hosts with overlapping territories must fail to provide the critical level of adult population necessary for primary tick establishment. Unusual wild fauna conditions, such as, for instance, a 'plague' of hares might occasionally tip the scale, but the writer finds that spread can invariably be traced to straying or deliberate moving of sheep in sufficient numbers. MacLeod's error in assuming the inevitable immediatism (implied) of marginal extension lay primarily in his failure to recognize the dominant role of sheep in the host-potential of rough pastures. A good sheep fence can protect a farm from neighbouring ticks, at least for a long period.

On the other hand, with no hindering fence, areas contiguous to infestation may remain without permanent tick population even of the lowest order, and that in hill country. Such areas escape because of their poor tick cover alone. For example, hill farms in Upper Coquetdale (Northumberland) are characterized generally by 'fine' herbage, becoming rather dry in a normal summer and liable to scorching in a hot drought; they are contiguous and unfenced on the heights with the 'rough' heavily infested grazings of Redewater Valley; yet despite sheep straying, the Upper Coquetdale farms remain uninfested (see Milne, 1944). One of those farms, Carshope, has had greater opportunity of infestation than that afforded by the straying of a few sheep. In May about 1925, three hundred ewes were driven from the heavily infested Southern Knowe farm in College Valley over to Redewater. The journey took 2 days and the infested flock rested during the intervening night on a small part (relatively the roughest) of one of the Carshope pastures. A few ticks were seen on the native sheep of that pasture at lambing the following year—for the first time in living memory—but by the next year they had disappeared and were not seen again. Where marginal cover is unsuitable, then, a focus of tick infestation cannot extend its boundaries (cp. §3, i, above).

The case of Carshope is paralleled annually on various lowland farms. To the latter in autumn come lambs or 'cast' ewes from the hill flocks for fattening or cross-breeding purposes. Sheep from areas with an autumn tick activity season (as in western Britain) must often bring ticks; but one hears nothing of permanent tick invasion of lowland pastures with their close-grazed open swards. Hence a new focus of infestation can only arise where cover is adequate (cp. §3, i, above).

It seems worth mentioning that plot 5 at Hethpool (see §2, iii) is somewhat similar in character to Carshope or Upper Coquetdale pasture in general. This plot yields a small number of unfed ticks of all stages every year. It does so almost wholly at the expense of the surrounding major rough area of the pasture. If plot 5 could be fenced off and still stocked at the same sheep density, its tick population would no doubt disappear after a year.

(c) *Infestation foci: the historical aspect of 'spread'*

The foregoing discussion begs the question why there are foci of infestation, i.e. why to-day are most rough areas infested but a few not? One would require to know very much more thoroughly the ancient history of the hills and moorlands—the changes in distribution of vegetational cover and hosts—before a precise answer could be given. One may perhaps surmise as follows:

It may be that conditions resulted only in isolated pockets of established ticks within the forests once clothing the hills and moorlands. The larger herbivorous mammals always make the greatest contribution to host-potential. In olden times, before the forests were cleared, there would be less grazing area with consequent lower general host-potential. The latter would result in a smaller proportion of the available ticks being able to feed and/or mate (see §2, ii) over the countryside as a whole. Furthermore, local host-potential would be unreliable since the larger wild herbivores would shift from place to place in their herds and flocks, moving on as they tired of or exhausted a pasture or as predators forced them, and perhaps not revisiting a pasture at the next activity time, but before then or later. The latter event would be fairly disastrous because if they fail to achieve a host, ticks do not remain active outside

the normal activity season (Milne, 1945*b*) and they die before the next season. Accordingly, *I. ricinus* would in those days lead a more precarious existence for the most part, perhaps establishing itself permanently only in host-favoured clearings with adequate cover, and constantly being dissipated over surrounding country less suitable as to terrain and/or hosts.

Since then great changes have taken place. To-day host-potential is much higher and more secure by comparison. It is higher because of unnaturally dense stocks of sheep. It is more secure because these sheep are confined to the pastures by fencing or hefting. Thus fewer ticks are left to starve, or miss the opportunity of mating, for want of a host at the right time. With the beginning of intensive sheep farming, tick populations in some existing pockets would build up to spread-favouring densities. With the gradual clearing of forests, spread would be facilitated by transference of sheep stocks to the new pastures; and perhaps by the movements of large wild mammals not yet inconsiderable in numbers nor greatly restricted in their ranges. Gradually the tick-infested areas would grow, not only because of the high sheep-host-potential available to make the most of chance introduction of a primary sufficiency of engorged ticks, but also because of progressively increasing areas of suitable ground. The latter process is still going on. Where geological conditions permit, hill and moorland pasture as a whole has been and is deteriorating more and more to the 'roughness' (see Department of Agriculture for Scotland, 1944) favouring ticks: cattle have practically disappeared where once they were more important than sheep; and the selective grazing of sheep has allowed the coarse, mat-forming bents to gain the upper hand. Bracken has encroached enormously and with increasing rapidity during the last 40 years. For lack of burning or a comprehensive policy of burning, heather has become rank and mossy at base. For economic reasons, drains have fallen into disrepair, thus encouraging rankness of vegetation. In short, land area suitable for tick survival and development has increased and is even to-day increasing. There can be little doubt that human activities have caused a great increase of tick densities and of infested areas in the last 500 years. Though lessened in these modern 'tick-conscious' times, both kinds of spread by sheep still take place through ignorance or accident. Marginal spread may be proceeding imperceptibly under occasional exceptionally favourable wild host conditions.

4. SUMMARY

Spatial distribution is understood to connote distribution of density (including zero density) over the land surface at a given time. Combining fact and conjecture, the following is a theory of spatial distribution of *Ixodes ricinus* in Britain. The tick passes practically the whole of its 3-year life span on the ground, spending a total of only 3 weeks on hosts. A considerable amount of vegetational cover is necessary for efficient survival on the ground; the more important limiting circumstances in lesser cover seem to be humidity (conditioned by temperature) in summer, perhaps temperature (alone) in winter, and the activity of predators. A host is necessary for mating as well as food purposes. Though small at best, the tick's chances of achieving a host are greater the more hosts on the ground. In practice this means the more sheep, since the latter are by far the main hosts. For the most part, spatial distribution to-day can be explained adequately in terms of interplay between amount of ground-cover and of host-potential, with cover the master factor. Thus poor cover keeps the heavily stocked lowland pastures free of permanent tick population even when opportunity of colonization is offered. The tick is confined to the hills and moorlands with their deep vegetation layers. There, within limits, it is more numerous on a pasture the more universal the distribution of adequate cover (because it drops at random from the host) and the higher the host-potential. Among hill and moorland pastures, however, there are some cases where, despite ample cover and host-potential, either (1) no ticks are present, or (2) the tick population is lower than it ought to be. These cases can be explained on grounds of lack of opportunity for colonization (1); or colonization so recent that population has not attained the level of 'dynamic equilibrium' with the environment (2). The question of 'spread' (colonization) is discussed fully in the text. The sheep flock is the main agent in spreading ticks. Spread is comparatively slow and relatively small in extent nowadays. There has very probably been considerable increase both in tick population densities and in infested areas since the advent of intensive hill and moorland grazing with farm stock some centuries ago.

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THE OCCURRENCE AND MORPHOLOGY OF *COENURUS SERIALIS* IN RABBITS

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(With Plates I–IV, containing Figs. 1–11)

INTRODUCTION

During an investigation into the effects of certain diet deficiencies in rabbits (carried out while I was on the Staff of the Royal Veterinary College) two does were accidentally infected with *Coenurus seriali*. The rabbit is usually infected by eating herbage which has been contaminated by a dog with the eggs of the tapeworm *Taenia serialis*, and it is assumed that this was the mode of infection in these rabbits. As will be seen later, this assumption is very important, because it makes it possible to state the approximate age of one of the primary cysts.

In the descriptions which follow, the word specimen refers to the parasite and not to the rabbit; all figures are actual photographs.

SPECIMEN NO. 1

This specimen was obtained from a rabbit which was born in the rabbitry on 14 December 1942 and died on 5 January 1945. Some time prior to her death this doe became emaciated and showed marked distension of her abdomen. It was suspected that she had some cystic or other pathological condition.

When the abdominal cavity was opened at the post-mortem a large cyst-like swelling was found retro-peritoneally on the dorsal abdominal wall dorsal to the left kidney which was displaced medially. The liver was markedly reduced, possibly as a result of the pressure of the cyst (Fig. 1). The ovaries were small and atrophic. After the peritoneum was dissected away an adventitious cyst of host tissue, containing thirty-seven coenuri, was found (similar to Fig. 2 which is specimen no. 2). Of these coenuri one (Fig. 3) was much larger than the others, being approximately 15 cm. in diameter and weighing 510 g. The thirty-six smaller coenuri, some of which were slightly elongated (Fig. 3), varied from approximately 0·5 to 5·0 cm. in diameter. As they were all contained within the same adventitious cyst, it would seem that they had all developed as the result of one infection. Also as the small coenuri contain scolices at a much earlier stage of development (Figs. 4, 5) than those of the large coenurus, it seems fairly certain that they are secondary external daughter coenuri which have been cut off from the large and therefore primary coenurus. This view is supported by the fact that the large primary coenurus (Fig. 3) shows diverticula which might be the beginning of more daughter coenuri. Also some of the secondary coenuri showed diverticula (Fig. 5).

Because of the known history of this doe it is possible to give the approximate duration of the infection. The doe had access to concentrates, sugar-beet pulp, green food and carrots until 1 April 1943 (i.e. up to the age of 16½ weeks). After this date she was fed on concentrates and sugar-beet pulp, which are not very likely to be contaminated, thus suggesting that infection occurred some time between 1 January 1943 (allowing 2 weeks before she would eat much food) and 1 April 1943. This would indicate that infection had taken place some time between 21 and 24 months before causing the death of the host. Also supporting the hypothesis that infection was of long duration is the fact that this doe would never take the buck. While it is fairly certain that the presence of the cyst was one factor contributing to the refusal to mate, it cannot be regarded as the only one, since the doe was deficient in vitamin A.

SPECIMEN NO. 2

This specimen was found in a doe which had been introduced into the rabbitry on 26 October 1942 as a fully grown rabbit which had had several litters. No other previous history is known. She continued to breed for some time, producing a litter of four on 14 December 1942, and another litter of eight on 2 May 1943. She mated on 2 July 1943, apparently successfully, and again on four different occasions prior to 3 October 1944, when she was killed, but never produced any more young. When the abdominal cavity was opened, at the post-mortem, a large cyst-like swelling was found in the same position as in the other rabbit. In this case the liver appeared normal but the left kidney was considerably compressed and flattened and was no longer kidney-shaped (Fig. 2). On dissecting away the peritoneum an adventitious cyst of host tissue was found (Fig. 2), which con-

tained one oblong primary coenurus measuring approximately 14 by 8 cm., and one small round coenurus having a diameter of approximately 1·3 cm. As in the case of specimen no. 1 it is thought that the large coenurus is a primary one and the other a secondary external daughter coenurus.

This doe was fed on concentrates, sugar-beet pulp, green food and carrots all the time she was in the rabbitry, so that infection could have occurred at any time, but it is very probable that it occurred at the same time as for specimen no. 1. This would suggest that infection had occurred some 18–21 months prior to the rabbit being killed. If this hypothesis is correct, and since only one secondary external daughter coenurus was found, as compared with thirty-six in the other case, it would indicate that the secondary external coenuri do not begin to develop in large numbers until the primary cyst has become fully mature, i.e. during the second year of infection (after approximately 18 months). Also it should be remembered that this doe was killed, whereas the other one containing specimen no. 1 died; had she been allowed to live longer more daughter coenuri might have developed.

As this doe was fed on a diet containing sufficient vitamin A, sterility in this case must, in the absence of any other evidence, be attributed to the presence of the coenurus infection.

COENURI

Both primary coenuri contained several hundred scolices each but no secondary internal coenuri. What at first appeared to be internal daughter coenuri proved to be old scolices (see below) which had developed vesicles in their 'neck' region. In specimen no. 1 the scolices in one part of the coenurus are arranged (Fig. 6) in multiple rows radiating from a common centre, while in other parts they are in groups which vary from eight to over 100. In specimen no. 2 the scolices are in groups which do not display any definite arrangement. This shows that the character which gives its name to the species is not constant, a fact which has also been noted by Clapham (1942).

A normal scolex develops as a hollow ingrowth from the germinal layer of the coenurus. At the distal end of its lumen hooks and suckers develop. In both my primary coenuri the majority of the scolices are approximately 8 mm. long (Figs. 6, 7, 8 and 11), which is much longer than those found in the secondary external coenuri. The distal ends, where the hooks and suckers are situated, are bent over and slightly enlarged to form a 'head'. The pedicel or 'neck' region is considerably elongated, the elongation being associated with the age of the specimen. However, a few of the scolices in the primary coenuri are much larger, as the lumen in the 'neck' region, close to the point of attachment to the germinal layer, has become distended to form a vesicle (Figs. 6, 7, 9, 10 and 11). Henry & Ciuca (1914), Coutelen (1929) and Clapham (1940*a*, *b*, in an allied species) have also observed vesicles which have developed from the 'neck' region of the scolices. When the vesicles first become noticeable they are 2 or 3 mm. in diameter but gradually increase in size. The largest vesicle found was 30 mm. in diameter. When the vesicle is between 4 and 5 mm. in diameter the whole scolex becomes detached from the germinal layer and is free within the coenurus. Sometimes groups of vesicular scolices adhere together (Fig. 11) after becoming detached from the germinal layer. Once detached the vesicle increases in size and becomes, at first, rather elongated, but ultimately spherical. It appears that the increase in size of the vesicle corresponds with a shortening of the 'neck' of the scolex. By the time the vesicle reaches 30 mm. the head of the scolex has become so reduced that it is just visible to the naked eye.

It would seem that this vesicle development commences approximately 18 months after infection, because vesicles are only just beginning to appear in specimen no. 2, while the largest vesicle (30 mm.) was found in specimen no. 1, which was between 21 and 24 months old. These vesicles, especially the large ones, contain a pinkish coagulated and slimy substance or debris, which, except for a few cells resembling phagocytic leucocytes, and a few crystals, displayed no definite structure. As large vesicles occurred in specimen no. 1, which caused the death of the host after 21–24 months, it appears that the development of these vesicles by the scolices is a sign of degeneration. Clapham (1940*a*) describes similar vesicles containing a pinkish debris in *Coenurus glomeratus*, but does not mention anything approaching 30 mm. in diameter.

Of the thirty-seven secondary external coenuri (thirty-six in specimen no. 1 and one in specimen no. 2), all, except the very small ones, contained one or more developing scolices (Figs. 4, 5). These very small and newly formed coenuri show areas little bigger than pin-points which are the sites of future scolices. That secondary external coenuri do not contain mature scolices when formed seems to be due to the fact that the diverticula from which secondary coenuri are formed develop from the wall of the primary coenurus where no scolices are present. Indeed, it would seem that the scolices in a secondary coenurus do not develop until it has been completely cut off from the primary coenurus.

The difference in the size of the scolices in my two primary coenuri and those in my secondary coenuri suggests that the main function of the secondary coenuri is to lengthen the life of the organism as a whole, thus enabling it to retain the power of giving rise to adult worms until the host (i.e. rabbit) is

eaten by the definitive host (i.e. dog etc.). This suggestion is supported by the fact that in specimen no. 1 there were thirty-six secondary coenuri, whereas in specimen no. 2, which, as far as I know, is 3 months younger, there was only one secondary coenurus.

With regard to the development of external daughter coenuri it should be noted that secondary external coenuri appear to have the power of giving rise to tertiary external coenuri by a similar development of diverticula, as in the case of the primary coenuri. This is shown in Fig. 5, which is a secondary external coenurus of specimen no. 1.

DISCUSSION

In the past it has generally been accepted that *C. serialis* produces secondary internal coenuri as well as secondary external coenuri (Neveu-Lemaire, 1936; Bonnal, Joyeux & Bosch, 1933; Brumpt, Duvoir & Sainton, 1934; Railliet, 1891).

The purpose of this paper is to bring forward proof that in at least two mature *C. serialis* no secondary internal coenuri ever appeared within the primary coenurus. That infection was of sufficient duration for secondary internal coenuri to develop, had they been going to do so, is obvious from the fact that specimen no. 1 had proceeded far enough (21–24 months) for thirty-six secondary external coenuri to develop and to cause the death of the host, while specimen no. 2 had proceeded far enough (18–21 months) for one secondary external coenurus to develop before the host was killed by the author. Also both my specimens had scolices which had developed vesicles. Henry & Ciuca (1914) state that the primary coenurus is mature and has started to develop secondary internal coenuri at the age of 6–8 months, therefore mine, which were 18–24 months old and had a capacity of approximately 500 c.c., had developed much longer than the minimum. Coenuri are generally regarded as being mature when they have reached the size of a hen's egg or a small apple (Coutelen, 1929, 1944; Railliet, 1891), although Henry & Ciuca recorded one of 800 c.c.

Henry & Ciuca describe how scolices develop vesicles, become detached from the wall of the coenurus, increase in size at the expense of the old scolex which is now replaced by numerous white spots, the future scolices of the daughter coenuri; these secondary coenuri, they say, remain within the primary coenurus. In my opinion Henry & Ciuca, and all authors mentioned above who describe secondary internal coenuri, have confused the adventitious cyst of host tissue with the primary coenurus, and therefore what they describe as secondary internal daughter coenuri within the primary coenurus should be described as one primary coenurus plus numerous secondary external coenuri within an adventitious cyst of host tissue. This error, in my opinion, has arisen by their comparing coenuri which contained vesicular scolices with older coenuri which have developed secondary external coenuri within the adventitious cyst of host tissue. I make this suggestion because not even my largest vesicle (30 mm. in diameter) of a scolex showed any sign of developing additional scolices. Coutelen (1946) and Clapham (1940*a*, *b*, in an allied species) also describe these vesicular scolices; Coutelen says that their number is in proportion to the age of the specimen, but makes no suggestion that they will ever develop into secondary coenuri. My opinion (which appears also to be that of Clapham) is that the development of a vesicle by a scolex is the beginning of degeneration, and under no condition does it give rise to a secondary internal coenurus.

From the evidence available the only secondary coenuri which ever develop are external to the primary coenurus but within the same adventitious cyst of host tissue. I therefore agree with Coutelen (1944–5, 1946) and others, that all the coenuri within one adventitious cyst are the product of one embryo.

SUMMARY

1. Two specimens of *Coenurus serialis* in the rabbit are described.

2. The duration of infection is estimated at 18–24 months.

3. Secondary external daughter coenuri had developed within the same adventitious cyst of host tissue as the primary coenuri.

4. No internal daughter coenuri were found within the primary coenuri.

5. Both primary coenuri contained some scolices which had developed vesicles (ranging from 3 to 30 mm.) in their 'neck' region. These vesicular scolices remain attached to the germinal layer until the vesicle is 4–5 mm. in diameter, when they become detached. These would appear to be the internal daughter coenuri of many authors but are here shown to be degenerating scolices.

I wish to thank Dr H. A. Baylis, of the British Museum (Natural History), for verifying the identification of the species, and for his help and criticism. The rabbits on which the observations were made were purchased and partly maintained by a grant from the Dixon Research Fund of the University of London. The photographs are the work of Messrs Burgess and Hancock, Histology Department, Royal Veterinary College.

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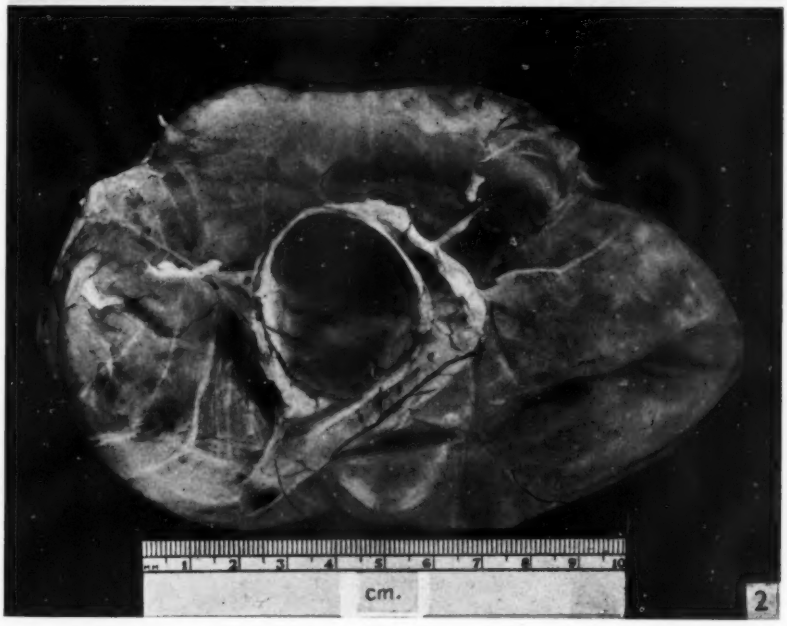
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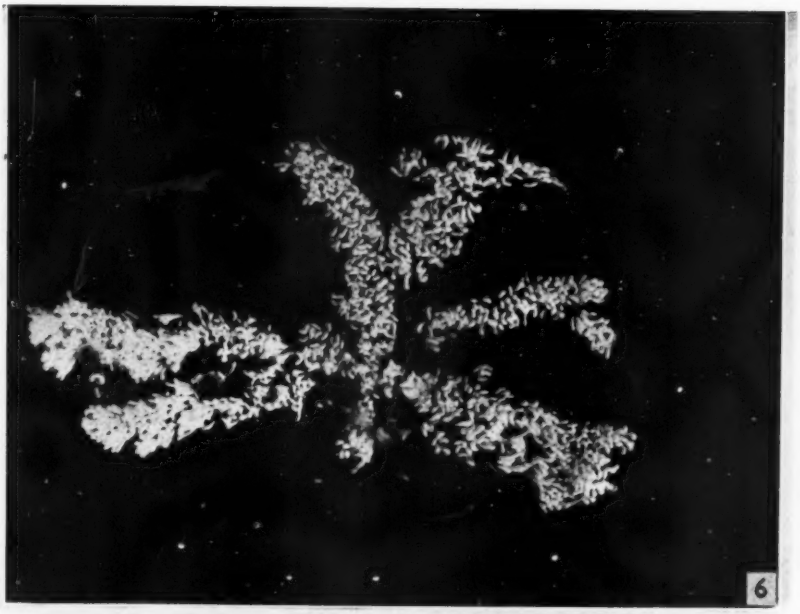

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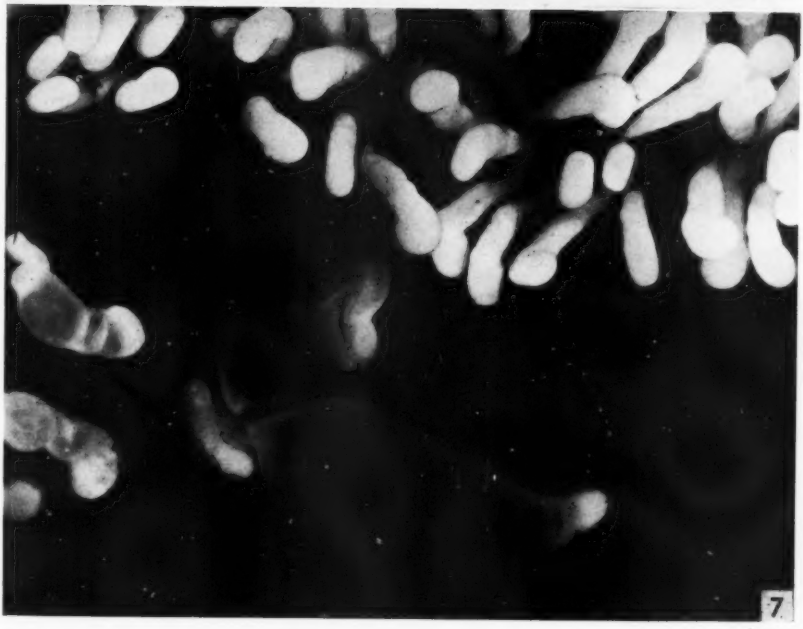

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EXPLANATION OF PLATES I–IV

PLATE I

Fig. 1. Dissection of rabbit showing specimen no. 1. *in situ* dorsal to the kidney and covered by the peritoneum. Note small and compressed liver.

Fig. 2. Specimen no. 2. Complete adventitious cyst of host tissue after removal from rabbit. Note compressed kidney, and a small portion of the peritoneum still adhering to the cyst.

PLATE II

Fig. 3. The primary coenurus and some of the thirty-six secondary external coenuri removed from the adventitious cyst. (specimen no. 1).

Fig. 4. × 10. A small secondary external coenurus (specimen no. 1). Note the row of developing scolices (bottom right) and group of developing scolices (top right).

Fig. 5. × 6. Secondary external coenurus further developed than coenurus in Fig. 4. Note scolices showing rings of hooks, also large diverticulum (bottom right) about to be cut off, and numerous small diverticula at bottom.

PLATE III

Fig. 6. × 1. Primary coenurus (large one in Fig. 3) opened to show serial arrangement of scolices. Note some scolices which have developed vesicles.

Fig. 7. × 6. Enlargement of portion of Fig. 6 to show scolices with vesicles.

PLATE IV

Fig. 8. × 25. Slightly elongated scolex removed from primary coenurus. Note lumen passing up neck region of scolex and curving round in head region where the hooks and suckers are situated.

Fig. 9. × 25. Scolex developing vesicle in 'neck' region. Note point of attachment and small amount of debris in vesicle.

Fig. 10. × 15. Scolex with large vesicle, now detached from wall of coenurus. Note that vesicle has developed at expense of the 'neck' (cf. Fig. 9).

Fig. 11. × 1. Portion of a primary coenurus (specimen no. 2) showing some slightly elongated scolices still attached and a group of vesicular scolices detached.

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THE HARVEST MITES ('AKIDANI') OF JAPAN AND THE FAR EAST AND THEIR RELATIONSHIP TO THE *AUTUMNALIS* GROUP OF TROMBICULID MITES

By CORNELIUS B. PHILIP and H. S. FULLER*

(With Plate V and 3 Figures in the Text)

The number is increasing of described species of trombiculid or chigger mites, whose larvae have a pentagonal scutum resembling roughly the classical European harvest mite, *Trombicula autumnalis* (Shaw). This mite was for a long time placed in the genus *Leptus*, and later in *Microtrombidium*. In 1925 Hirst erected a new subgenus, *Neotrombicula*, for which *autumnalis* is monotypic, though the name has not been generally adopted. This antedates *Pentagonella* proposed by Sig Thor (1936) for *Trombidium ardeae* Trägårdh, 1904, genotype, and five other species, though it is not at all certain at present that Thor's genus is strictly synonymous with the preceding as discussed later. If the peculiarly elongate nymphal and adult stages reared and/or figured by Okumura (1918) and Kawamura (1926) for the Japanese harvest mites are found in other species of this group as well, it may be necessary eventually to revive *Neotrombicula* Hirst. In the meantime, the species discussed below will be retained provisionally in the genus *Trombicula* sensu lato.

Incidental to his early studies of the 'kedani' or 'akamushi' vectors of tsutsugamushi disease in Akita Prefecture of northern Honshu, Japan, Tanaka was the first to call attention to the occurrence on local voles (*Microtus*) of mites that resembled the European harvest mite, '*Leptus autumnalis*'. He and several other Japanese students later referred to this as the 'Japanese *Leptus autumnalis*', either entirely in Japanese script, or with the last two words only in Latin. Owing to inaccessibility of certain Japanese articles, recent systematic literature (e.g. Womersley & Heaslip, 1943) has followed Tanaka, Kaiwa, Teramura & Kagaya (1930) in crediting Tanaka (1916 and 1918) with the specific name *japonica*, though as pointed out by Philip (1947), the first acceptable nomenclatorial use of this name actually dates from Tanaka *et al.* (1930) as *Trombicula autumnalis japonica*.

These so-called Japanese harvest mites were also referred to by various authors (Okumura, 1918 Nagayo, Miyagawa, Mitamura & others, 1921) as 'akidani', and Miyajima & Okumura (1917) as well as Kawamura (1926) also mistakenly compared a Formosan species with these. Probably this Formosan species was the same as that referred to by Kawamura & Yamaguchi (1921, p. 186) as 'Leptus autumnalis formosanus', printed without italics, and as '*Trombicula mediocris* (?)' adults. It was called '*T. pseudo-akamushi* non Tanaka' by Hatori, and was given the new name by Womersley & Heaslip (1943)—*T. hatorii*; in all probability it is actually *Eutrombicula wichmanni* (Oudemans, 1905), as discussed by Philip (1947).

As a result of field activities for the United States of America Typhus Commission by one of the authors in Burma (Mackie, Davis, Fuller and others, 1946), and by the other in Japan (Philip, 1947), it has been possible to add the following observations to this group of mites. Measurements, where unstated below are in μ.

Trombicula japonica Tanaka *et al.* 1930
(Text-fig. 1)

This is a species of the above group of chiggers with pentangulate scutal shield in the larva, and it is further readily distinguished from related species by the combination of two pairs of humeral setae (i.e. ds 1 = 4) (Fig. 1C), and one long, nude, outstanding bristle or seta, situated dorso-basally on the last hind-tarsal segment (Fig. 1D). The sensillary hairs or pseudo-stigmatic organs have only four to five barbs confined to the middle third with the base and apex bare. Details of larval characters of *Trombicula japonica* are provided in Fig. 1, standard data in Table 1, and setal characters in Table 2. The galeal setae are usually nude, sometimes forked. The dorsal and lateral setae on palpal segment IV (tibia) are sometimes nude, but usually forked or with 3-4 branches in varying combinations. There are two types of feathered setae on the legs: one short, usually curved, and barbed on the outer (convex) side, the other long with barbs on both sides.

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This species, under one common name or another, has been reported and/or figured from one or more of the three prefectures of Akita, Niigata, and Yamagata by Tanaka (1918), and Kawamura (1926), and is said to be bright red in life. It is reported to occur in Niigata only in November and December, but in Akita from the end of September through December. Specimens taken by Philip (1947) in October in Yamagata and Niigata were found to be more frequent on the belly of both *Apodemus* and *Microtus* than in the ears, though Tanaka reports it only from the ears of voles. Its propensities for attacking man are unstated, and its relationship to the natural maintenance of tsutsugamushi disease is unknown.

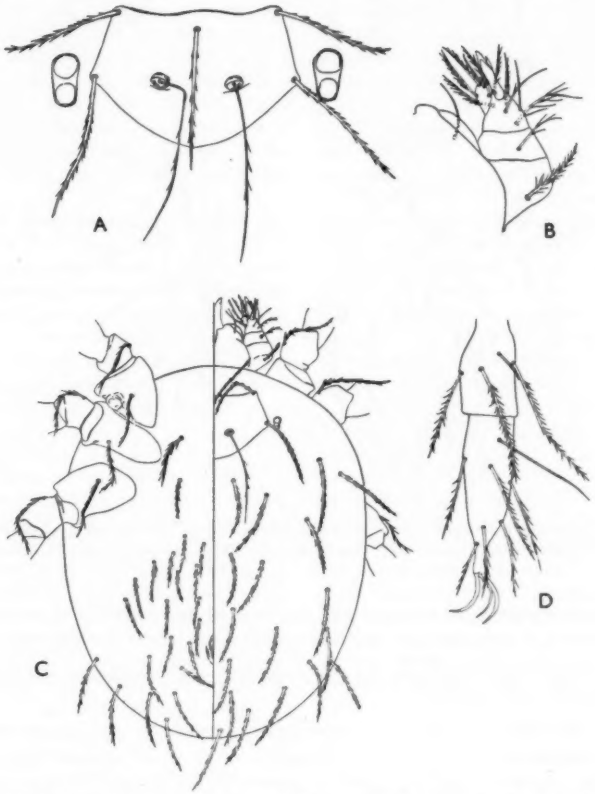

Fig. 1. *Trombicula japonica* Tanaka *et al.* A. Scutum and eyes. B. Galeal and palpal setae. C. Ventral and dorsal body vestiture. D. Terminal segments of hind leg.

Trombicula tamiyai n.sp. (Text-fig. 2)

In addition to *Trombicula japonica*, there has been figured by several Japanese workers (Okumura, 1918; Kawamura, 1926) a second species of 'akidani', which was recognized as distinct by some and apparently confused by others. No scientific name has been applied to this species. The authors take pleasure in dedicating it to Takeo Tamiya of Tokyo Imperial University, who has been especially helpful in assisting us in supplying specimens and inaccessible literature on Japanese mite taxonomy. His co-authorship of many reports of tsutsugamushi disease and its vectors, particularly of studies in Yamagata Prefecture, is well known. Nagayo *et al.* (1921) report this species as occurring 'on the ear lobes of voles' in Yamagata in maximum numbers in November and sparingly from mid-October to May. It is probable that some of these records actually refer to *T. japonica*. As his species E″, Kawamura (1926) records *tamiyai* in Niigata from the end of October through May.

The larva is readily distinguished from that of *T. japonica* by the more rounded, deeper, but shorter-sided scutum, with the sensillary pores situated about midway between the fore- and extreme hind-

margins, and behind the line of pl (Fig. 2A), by the branched galeal and palpal setae, the single humeral seta on each side (ds = 2), and the different standard data (Table 1) and setal characters (Table 2). The sensillae have more barbs.

Colour in life 'deep red' according to Nagayo *et al.* (1921). Engorged larvae are ovoid, approximately 256 wide by 416 long. Striae normal, not crenulate.

Galeal setae and all palpal setae plumose or

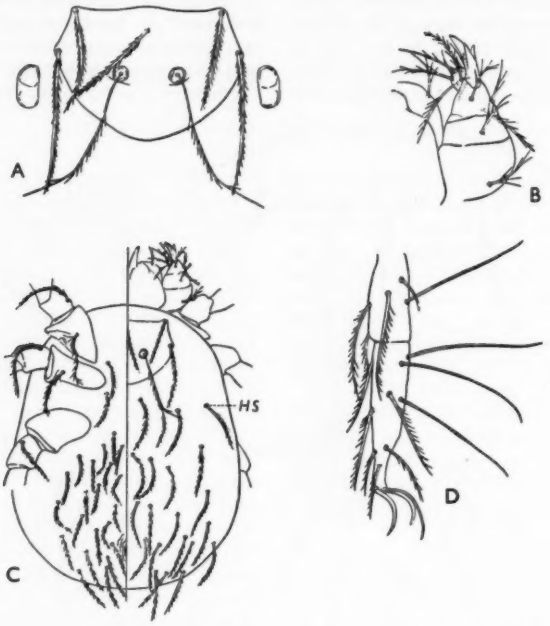

Fig. 2. *Trombicula tamiyai* n.sp. A. Scutum and eyes. B. Galeal and palpal setae. C. Ventral and dorsal body vestiture. D. Terminal segments of hind leg.

Table 1. *Standard data in microns of scutal and setal measurements of* Trombicula japonica *compared to* T. tamiyai *n.sp. and* T. fujigmo *n.sp. Observed range and mean.*

Abbreviations after Womersley; lower case denotes respective setal lengths; hs = humeral setae, dsa = dorsal setae anterior, vsa = ventral setae anterior.

Species ...	*japonica*		*tamiyai*		*fujigmo*		sp.
Locality ...	Honshu		Honshu		North Burma		East India
No. specimens ...	17		31		18		1
AW	72–82	79·3	60–69	64·5	57·5–64	60·6	76
PW	91–98	96·3	75–88	82·2	77–86·5	80·7	100
SB	29–36	31·9	24–31·7	27·3	25–28·8	27·4	30
ASB	30–43	35·7	27–30	28·3	26–29	28·0	30
PSB	27–32	29·4	30–36	32·6	28·8–32	30·8	34·5
SD	58–75	65·0	57–66	70·0	56·8–61	59·0	64·5
AP	30–34	32·0	20–25	21·5	22·5–29	27·2	27
am	56–65	61·2	42–48	44·5	35·2–48	41·7	58
al	52·5–63	57·3	42–51	48·0	29–40	36·6	46
pl	66·5–81	71·6	58–68	62·5	41·6–54	46·4	58
sens	88–94·5	91·5	70–85	76·0	64–80	72	87
hs	67–73·5	72·6	51–60	54·1	43–51·2	46·0	—
dsa	54·4–77	—	45–60	—	38·4–42	—	48
vsa	30–48	—	26–35	—	25–33	—	30–36

branched (Fig. 2B), except the usual sensory rods on segment V(the 'thumb'). Palpal claw trifurcate.

Legs with coxae unisetose, and the usual vestiture of branched setae. Tarsus III more slender than in *japonica*, and bearing three long, upright, slender, nude setae. A similar one on tibia III (Fig. 2D).

Scutum (Fig. 2A) with the usual setae long and prominently ciliated, the anterior median a little shorter than the anterolateral pair, the posterolateral pair the longest (measurements in Table 1). Sides convergent anteriorly and very short, the sensillary bases behind the line of the posterior lateral angles and a little in advance of the middle between the fore, and hind-margins. Hind-margin extended behind and rounded with a very slight median angle. Sensillary hairs (pseudostigmatic organs) long, flagelliform and with moderately dense barbs on about the distal three-fourths except the extreme apex.

Dorsal setal count in rows somewhat variable: 2-(8 to 12)–(8 to 11)–(8 to 12)–(6 to 9)–(4 to 6)–2, total 43 to 56. In engorged specimens, the humeral setae and the outer bristle at each end of ds row 2 are often displaced caudad giving the impression of no hs (Fig. 2C). Posterior rows irregular with adventitious lateral setae making exact counts difficult. Total ventral setae 41–50, shortest in the preanal group of approximately 30–34, excluding the two sternal pairs.

Data on holotype larva: ds: 2–10–8–2–9–2–6–4-2=45; length of humeral setae 51·2, of outer ds row 2–48; vs count: preanal 34, post-anal 12, total 46. AW 65·6, PW 80, SB 25·6, ASB 28·8, PSB 30, SD 58·8, AP 19·2, am 48, al 42·5, pl 60·8, sens 73·6. Okiage village, Yamagata Prefecture, Honshu, Japan, 17 September 1920. Host, probably *Microtus montebelli*. U.S. National Museum, no. 1781.

Holotype and twenty-one paratypes, same collection data; two paratypes, 18 November 1920, and one paratype, 19 November 1920, same locality data; three paratypes, 22 October 1920, Arato, Yamagata (given us by Dr Takeo Tamiya, and remounted on separate slides by Mr H. Womersley). In collections of the U.S. National Museum, British Museum (Natural History), Rocky Mountain Laboratory, South Australia Museum, Dr G. W. Wharton, Dr Takeo Tamiya, and the authors.

Original slides from Dr Tamiya were labelled with the Japanese 'kanji' character which refers to 'woodcutter' or 'woodman', suggesting an association with 'woodcutter's itch' and a propensity for attacking man.

Trombicula fujigmo n.sp. (Text-fig. 3)

Among the chigger mites taken during field operations in north Burma by the U.S.A. Typhus Commission in 1944–5, was a series of eighteen specimens which were at first thought to be the same as the preceding *Trombicula tamiyai*. These also have three long nude setae on the hind tarsi and another on the metatarsi, and the shape of the scutum is casually similar.

The most striking difference is in the branched condition of all the palpal and the galeal setae in *T. tamiyai*; in *T. fujigmo* the galeal, and the dorsal and lateral setae on palpal tibiae (the fourth or 'thumb-bearing' segment), are nude or at most occasionally forked. In addition, there are significant differences in certain of the standard data, particularly of the distance between the bases of the antero-lateral and postero-lateral setae (AP) on the scutum. In *tamiyai* the sensillae have more tendency to be situated slightly behind the line of pl as correlated with the shorter AP (Fig. 2) and to terminate in a longer, bare apex.

Setal data (Table 2) and lengths also differ. There are more and longer body setae in *tamiyai*. Of the scutal setae, am are shortest in *tamiyai*, while al are shortest in *fujigmo*. The ds counts in *fujigmo* vary somewhat: 2–8 (9)–8 (9)–2–8 (7)–4–2, with not as much displacement observed postero-laterally as in *tamiyai*; vs (excluding the two sternal pairs) total 28–36 with 21–25 in the shorter pre-anal group, and 11–14 larger post-anal setae.

Colour in life unknown. Engorged larvae are ovoid approximately to 410 × 295. Striae normal, not crenulate.

Galeal and dorsal and lateral setae of palpal segment IV nude (Fig. 3B). Palpal claw trifurcate. Legs with vestiture (Fig. 3D) as in *T. tamiyai*, but tarsus III proportionately longer and less chunky. Scutum as characterized in Fig. 3A and Table 1, sides convergent anteriorly, hind-margin broadly rounded, SB nearly in the midline, and on line of pl.

Data on holotype larva: ds: 2–8–8–2–7–8–4–2=41; length of hs 44·8, of outer ds row 2–41; vs count: pre-anal 23, post-anal 9, total 32; lengths, 32–38. AW 57, PW 80, SB 26·5, ASB 25·6, PSB 32, SD 57·6, AP 28, am 41, al 38·4, pl 44·8, sens 80. U.S.A.T.C. no. 939–4, 12 miles north of Myitkyina, Burma, 14 April 1945. In U.S. National Museum, no. 1499. Host, shrew, *Crocidura vorax* Allen.

Paratype larvae, one each per slide. Six, same U.S.A.T.C. data as holotype; two, U.S.A.T.C. no. 748 6 miles north of Myitkyina, Burma, 10 March 1945, from same host; three, U.S.A.T.C. no. 747, same locality data, from *Rattus rattus sladeni* (Anderson); four, U.S.A.T.C. no. 893, same host and locality, 29 March 1945; one U.S.A.T.C. no. 797, same host and locality, 21 March 1945; one, U.S.A.T.C. no. 957, Myitkyina, Burma, March 1945, from 'rat'. Paratypes deposited in same collections as those of *Trombicula tamiyai*.

The specific name *fujigmo*, commemorates a humorous, slang term evolved by soldiers of the

Allied Forces in the Far East to express their impatience to return home after V-J Day.

A single larva of what appears more likely to represent a distinct, though similar species rather than a variety of the above, was taken at Kanglatongbi, Manipur State, India, 6 December 1945, on *Rattus rattus brunneusculus* by Lt.-Colonel J. R. Audy, R.A.M.C., and staff of the Scrub Typhus Research Laboratory, South-East Asia Command, British Army.

General characters essentially as for *Trombicula fujigmo*. Scutal (Table 1) and setal data indicate significant differences in certain measurements, particularly as regards the much larger size of the scutum. Scutal measurements are greater for all characters than the maxima observed for *fujigmo* except AP. There are also more body setae (ds = 2, 12–10–8 + 15) (vs = 56). More specimens of this and *T. fujigmo* from other localities are needed to establish extent of variation and specific limits.

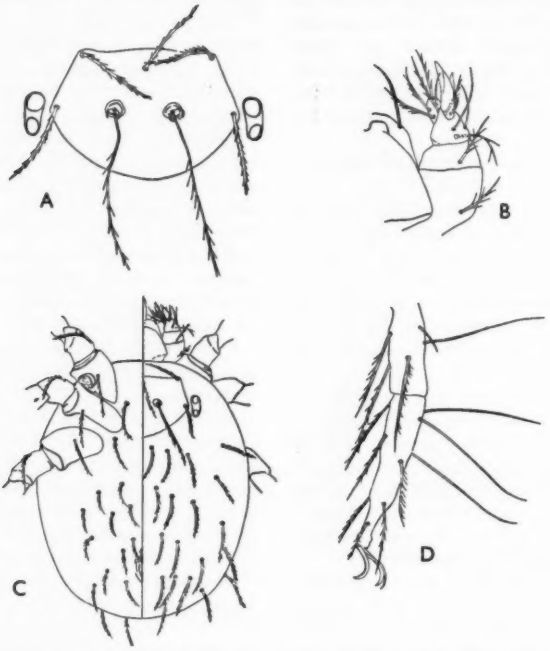

Fig. 3. *Trombicula fujigmo* n.sp. A. Scutum and eyes. B. Galeal and palpal setae. C. Ventral and dorsal body vestiture. D. Terminal segments of hind leg.

COMMENT ON POST-LARVAL STAGES

It appears that both species of Japanese harvest mites have been carried beyond the larval stage in the laboratory. Tanaka *et al.* (1930) figure (see Pl. V, Fig. 2) and describe a nymph reared from the larva of *T. japonica* (Kawamura's E′) which is more elongate than the usual *Trombicula* nymph. The adult is not certainly known.

Similar nymphs were also reared and figured by Kawamura (1926, E″) (see Pl. V, fig. 2) and by Nagayo *et al.* (1921), which, according to the descriptions of the associated larvae, were *T. tamiyai*, although the nymphs were not described in detail. The E″ adult reared and photographed by Kawamura is similar in its peculiarly elongated shape to an adult captured in Niigata and drawn (Pl. V, Fig. 1) by Okumura (1918), and which Nagayo *et al.* (1921) also mention rearing, but without description. However, Kawamura confused both his E″ with the Akita harvest mite of Tanaka (which is presumably *T. japonica*, judging by Tanaka's 1930 discussion) as well as his E′ (*T. japonica*) with the Taiwan harvest mite of Miyajima & Okumura (1917). This last was probably *Eutrombicula wichmanni*, discussed previously.

If Kawamura's figured nymph and adult were correctly associated with his E″ larvae (*Trombicula tamiyai*) in Niigata, then the less elongated, captured adult male (the penis in their figure indicates sex) illustrated by Nagayo *et al.* (1921) (see Pl. V, fig. 1) actually may have been *T. japonica*, which also

occurs in Yamagata, though the larvae they report rearing to similar elongated nymphal and adult stages were *T. tamiyai*. Further rearings will be necessary to clarify the biologies of these two Japanese species. But it is evident that one or both species are more closely related to *T. autumnalis* of Europe (as the Japanese previously recognized) than to *Trombicula* sensu lato.

The nymph of *T. autumnalis* (reared by Hirst and refigured by Warburton, 1928) has an elongate form, resembling figures of the shape of the Japanese species (Pl. V, fig. 2), suggesting that the long-sought adult of the European harvest mite should also have a similar elongated shape. A mite was figured as the adult of *T. autumnalis* by André (1930). It was captured in a circumscribed area of ground where the larvae were very common, but it was not associated with larvae by rearings; consequently it must be accepted with reservation. Through the kindness of Mr Kenneth L. Cockings, formerly of the Department of Entomology, London School of Hygiene and Tropical Medicine, we have been privileged to examine nymphs and adults of *T. autumnalis*, which have been associated by him with the larvae during the course of rearing experiments. These nymphs and adults from larvae collected in Great Britain are of peculiar elongate form. If similar biological data are forthcoming for related species, eventual generic or subgeneric recognition appears to be justified. However, complete application of *Neotrombicula* to known species will be difficult at present because of insufficient rearings of various species to correlate the presumed related larval characters, especially in view of the variation in strict pentangulation of the scutum previously noted. It appears likely that any present limits accepted for the so-called harvest mite group would have to be highly artificial, and the value of generic separation at this time appears dubious until much additional information is obtained. At best, it can only be stated at present that one or both Japanese species could be assigned to *Neotrombicula* with *autumnalis*. Assignment of other species based on larva alone is pure conjecture, but it appears desirable for the future monographer to present data on probable related species.

DISCUSSION OF LARVAL CHARACTERS

Not all chigger mites whose larvae have pentangulate scutums and flagelliform sensillae are necessarily related to the *autumnalis* group of *Trombicula* sensu lato, especially in the absence of biological data discussed above. For example, Michener (1946) has proposed the subgenus *Megatrombicula*, type *Trombicula alleei* Ewing, 1926, including also *T. attenuata* Michener, 1946, and *T. velascoi* Boshell & Kerr, 1942, with similar shaped scutums. Michener included a fourth species, *T. peruviana* Ewing, 1926, based on the adult, larva unknown, though possibly described under another specific name.

One of us (H. S. F.) has studied specimens of most of the species referred by Thor (1936) to *Pentagonella*, and it has become apparent that his emphasis on a pentagonal scutum encouraged him to bring together several species, not all of which we consider to be related systematically. The genotype, *P. ardea* (Trägårdh, 1904) and *P. acuscutellaris* (Walch, 1922), are closely related to each other and they share many characters with the known larvae of Michener's subgenus. The scutum of all these species is more acutely angulate posteriorly than the group under consideration, and they form a systematic group which is probably distinctive, yet it is impossible at this stage to cite key characters for their group separation from *Trombicula autumnalis* and *T. japonica* without at least post-larval stages for comparison. *Pentagonella fahrenholzi* (Oudemans, 1910) cannot be discussed here, as we have not seen specimens, and since the tarsi were missing from Oudemans' type specimen, the existence of nude, sensory setae on the hind tarsi is unknown. *P. trägardhi* (Oudemans, 1910) and *P. muris* (Oudemans, 1910) are known only from larvae, and in both species the scutum is more acutely angulate posteriorly than in *Trombicula autumnalis*, though there is a long, nude seta on tarsus III of each. These two species are distinct from *Pentagonella ardeae* and *P. acuscutellaris*, in that the latter two species have larger scutums which project farther posteriorly, and the dorsal setae are much longer than in *P. trägardhi* and *P. muris*, but again these differences may be of specific rather than generic significance. Finally, *P. yorkei* (Sambon, 1928) does not possess a long, nude seta on tarsus III, and it is doubtful if it is related to *P. ardeae* or to *Trombicula autumnalis*; furthermore, the palpal claw is bifurcate, while in the above-mentioned species it is trifurcate.

Certain other species with a pentagonal scutum have no long, nude setae on tarsi III, and although they have flagelliform sensillae, comparisons of actual specimens with *T. autumnalis* larvae suggest that they are not even closely related to this species. Examples of such species are *T. munda* Gater, 1932; *T. spicea* Gater, 1932; *T. cynos* Ewing, 1937; *T. cynictia* Radford, 1942; *T. mastomyia* Radford, 1942; *T. punctata* Boshell & Kerr, 1942; and *T. frittsi* Wharton, 1945.

The remainder of species with a roughly pentagonal scutum have the posterior margin more or less rounded, as in *T. autumnalis* and *T. tamiyai*, the palpal claw is trifurcate, and they possess one or more long, nude setae on the hind tarsae. In addition, there may be such nude setae on certain other segments of the third leg, but these have never been observed on legs I or II. In two members of this group, *T. rohweri* Ewing, 1942 and *T. californica*

Ewing, 1942, the sensillae are not barbed or ciliate, but nude. Possibly this character will be noted in other species and even perhaps lead to further subdivision.

Pertinent available data on some larval species which share characters with *T. autumnalis* are presented in Table 2 in tabular form. The two species marked with an asterisk (*) have not been seen by us, data being taken from the literature. Setae listed as nude are sometimes forked on one or both sides of the same specimen, and in *T. japonica* they are especially variable on palpal segment IV. Data on ventral setae on palpal segment IV are not tabulated, as these setae are branched on all species listed, except in *harperi* and *abyssinica*, in which they are nude. *T. parkeri* Radford has been omitted from the table as it is to be synonymized with *microti* Ewing in a future paper by Wharton & Brennan.

Table 2. *Data on larvae of species possibly related to* Trombicula autumnalis

Species	Leg III, long nude setae			Galeal seta	Seta, palp. seg. II	Seta, palp. seg. III	Setae, palp. seg. IV	
	Seg. IV	Seg. VI	Seg. VII (tarsus)				Dorsal	Lateral
Autumnalis (Shaw)	0	0	1	Nude	Bran.	Bran.	Nude	Nude
Japonica (Tanaka *et al.*)	0	0	1	Nude	Bran.	Bran.	Nude	Nude
Centropodis (Ew.)	0	0	1	Nude	Bran.	Bran.	Nude	Nude
**Guineense* (Bruy. & Joy.)	0	0	1	Nude	Bran.	Bran.	Nude	?
Trägardhi (Oud.)	0	0	1	Nude	Bran.	Bran.	Bran.	Nude
Muris (Oud.)	0	0	1	Nude	Bran.	Bran.	Bran.	Nude
Nigeriensis Ewing	0	0	1	Bran.	Bran.	Bran.	Nude	Nude
Desaleri Methl.	0	1	1	Nude	Bran.	Bran.	Nude	Bran.
Tamiyai n.sp.	0	1	3	Bran.	Bran.	Bran.	Bran.	Bran.
Fujigmo n.sp.	0	1	3	Nude	Bran.	Bran.	Nude	Nude
Shannoni Ewing	0	1	3	?	Bran.	Bran.	Bran.	?
Harperi Ewing	1	1	2	Nude	Nude	Nude	Bran.	Nude
Microti Ewing	1	1	2	Bran.	Nude	Nude	Bran.	Nude
Abyssinica Radford	0	0	1	Nude	Bran.	Bran.	Nude	Nude
Rohweri Ewing	1	1	2	Nude	Bran.	Bran.	Bran.	Bran.
Whartoni Ewing	1	1	2	Nude	Bran.	Bran.	Bran.	Bran.
Californica Ewing	1	1	2	Nude	Nude	Nude	Bran.	Nude
**Fahrenholzi* (Oud.)	0	?	?	Nude	Bran.	Bran.	Nude	Nude
Bisignata Ewing	0	1	3	Nude	Bran.	Bran.	Nude	Nude

Trombicula, then *Neotrombicula* Hirst would apply. Specific characters for other apparently related species are tabulated and discussed.

SUMMARY

Trombicula tamiyai and *T. fujigmo* n.sp. are described and figured from Japan and northern Burma respectively; the holotypes are in the U.S. National Museum. The first, unnamed or referred to European

Thanks are especially extended to Mr H. Womersley, of the South Australia Museum, for his interest and suggestions in this study, to Mr K. L. Cockings of the Institute for Medical Research, Malaya, for providing reared post-larval stages of *T. autumnalis*, and to Dr Takeo Tamiya, Dean of Tokyo Imperial University Medical School, and Colonel Crawford E. Sams, M.C., Chief, Public Health and Welfare Section, G.H.Q., Tokyo.

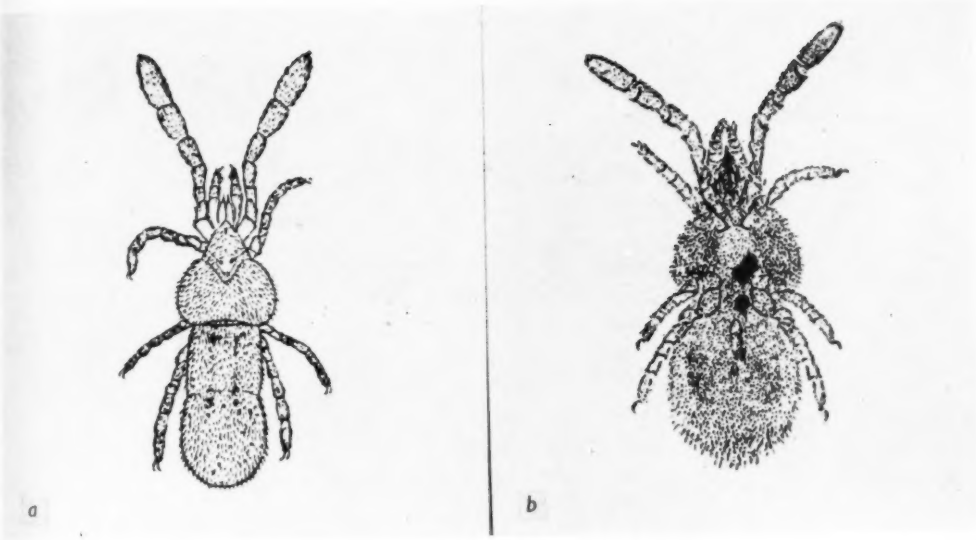

Fig 1.

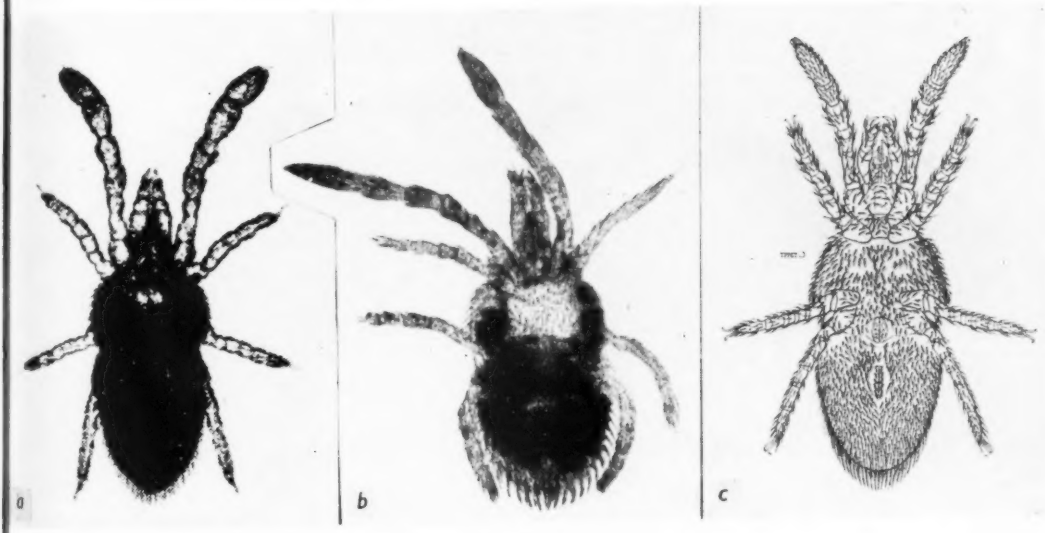

Fig. 2

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EXPLANATION OF PLATE V

Fig. 1. Possible elongated adults (wild-caught) of *T. tamiyai*: (*a*) Niigata, after Okumura (1918); and (*b*) Yamagata male after Nagayo *et al.* (1921).

Fig. 2. Comparison of forms of reared nymphs of (*a*) *T. tamiyai* (Niigata E″ of Kawamura (1926), (*b*) *T. japonica* (Akita, after Tanaka *et al.* (1930) and (*c*) *T. autumnalis* (after Hirst in Warburton (1928)).

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EXPERIMENTAL DIFFERENTIATION OF *LEISHMANIA*

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It is generally accepted that the various species of *Leishmania* of men and dogs are morphologically indistinguishable. Therefore it has been argued that, according to the present rules of taxonomy, they should all be included in one species, *L. donovani* (Laveran & Mesnil, 1903), Ross, 1903. A review of the literature indicates, however, that this view is not generally acceptable, while strict adherence to the morphological rule would require the inclusion of the *Leishmania* of lizards (*L. hemidactyli*, *L. agamae*, etc.) in the same species as those of men and dogs.

For one reason or another, numerous species and varieties have been created for the *Leishmania* of men and dogs (Kirk, 1948). The only criteria of differentiation which have so far stood the test of time are purely medical, and are provided by the clinical picture and geographical distribution of the cases from which the parasites were obtained. Numerous attempts to differentiate the species of *Leishmania* by the techniques of bacteriology have given extremely conflicting results in the hands of different workers. During the last fifty years, however, the infections produced by these parasites have been under practically continuous observation, and have been intensively studied. For a unicellular organism like *Leishmania* this is a long period, covering the lives of innumerable generations. Since clinical differences in the types of infection occurring in different areas have persisted during this period we are justified in concluding that they are the result of hereditarily stable differences in the parasites concerned. The medical records thus reveal important physiological or biological differences between different strains or races of *Leishmania*. Hoare (1943) has emphasized that, as regards hereditary stability of characters, biological races in Protozoa do not differ from morphologically distinguishable systematic units with constant characters.

In a previous communication the writer (Kirk, 1948) suggested the possibility that the differentiation of 'biological races' of *Leishmania* on the grounds of more or less well-defined adaptation to specific insect vectors would be consistent with the views of modern authorities on systematics. It is consistent also with conclusions derived from a study of the geographical distribution of *Leishmania* infections in men and dogs. This method would probably require the recognition of more (intraspecific) groups than are required by medical criteria. For example, Palestinian strains of oriental sore might be differentiated from those of Greece, Baghdad and India on the grounds that they are specifically adapted to *Phlebotomus papatasii*, whereas the others are adapted to members of the *sergenti* group. Similarly, strains of Indian, Chinese, Mediterranean and Sudan kala azar might be differentiated on the grounds of adaptation to different vectors, and further evidence in favour of this differentiation is provided by differences in the clinical features of the diseases caused by these parasites and in their reaction to chemotherapy. The recognition of biological races of the *Leishmania* of men and dogs on the grounds of adaptation to specific vectors would not be inconsistent with the experience of those who have studied Leishmaniasis clinically. There is evidence that the biological races of *Leishmania*, thus differentiated, could be grouped into a few higher categories corresponding roughly with the *L. donovani*, *L. tropica* and *L. brasiliensis* of medical text-books.

Attempts to differentiate *Leishmania* by the techniques of bacteriology have given extremely contradictory results (Kirk, 1948). Nevertheless, Russian workers (cf. Hoare, 1943) have reported findings which indicate that in Middle Asia there occur two very distinctive types of oriental sore which can be thus differentiated. It is possible that much of the confusion associated with the laboratory differentiation of *Leishmania* arises from the fact that many workers, especially in the early days, have not paid sufficient attention to matters which are now realized to be of fundamental importance in bacteriological technique, such as the fallacy of not differentiating between results obtained with freshly isolated strains and those obtained with strains which have been maintained in the laboratory for long periods and may have undergone variation in antigenic, cultural, or even invasive properties towards different hosts. Modern workers appreciate the importance of defining as clearly as possible the species and strains used in biological experiments. It has been reported in several recent papers that biological differences can be demonstrated between

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Table 1. *Biological differentiation of* Leishmania

	L. donovani	*L. tropica*	*L. brasiliensis*	Author
Character of growth in Adler's medium	Granular masses of flagellates standing out in a background of less dense growth	Flagellates uniformly diffused throughout the medium	—	Adler, 1947
Action of bile on flagellates	Rapid lysis	Rapid lysis	Flagellates resistent to action of bile	Senekji & Zebouni, 1941
Inoculation of Syrian hamsters (*Cricetus auratus*)	Visceral infection	Visceral infection	Local skin lesions only; visceral infection not produced even by intraperitoneal inoculation	Adler & Ashbel, 1940; Fuller & Geiman, 1942
Inoculation of Spermophils (*Citellus citellus*)	Intense visceral infection	Cutaneous lesions only; visceral infection not produced even by intraperitoneal inoculation	—	Blanc & Caminopetros, 1930, Caminopetros, 1936
Morphology of leishmanial forms in experimental animals	Parasites uniform in size ($4{\cdot}2\mu \times 2{\cdot}8\mu$); no large forms	Large forms up to ($5{\cdot}6\mu \times 3\mu$) in hamsters, white mice and spermophils	—	Adler, 1929, 1947, Caminopetros, 1936
Vectors	Usually sandflies of *major* group	Sandflies of *sergenti* and *papatasii* groups	Neotropical sandflies	Various

different strains of *Leishmania*. There is some evidence from these reports that the differentiation of *Leishmania* into three groups, as previously suggested (Kirk, 1948), corresponding to *L. donovani*, *L. tropica* and *L. brasiliensis* may yet be supported by biological differences which can be demonstrated in the laboratory by the techniques of bacteriology, using modern methods. The results are summarized in Table 1. As some of the observations still require confirmation, the data presented in this table are quoted, without prejudice, from the authors named in the last column.

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A NEW SPECIES OF PARASITIC MITE, *CNEMIDOCOPTES JAMAICENSIS*, A CAUSATIVE AGENT OF SCALY LEG IN *TURDUS AURANTIACUS*

By F. A. TURK, Ph.D., F.R.E.S., F.Z.S.

(With 6 Figures in the Text)

The specimens of this new species of mite were collected by Mr Gordon Thompson, formerly curator of the Science Museum of the Institute of Jamaica, from the legs of a specimen of the golden thrush, *Turdus aurantiacus* Gmelin, taken at Morant Bay Road, 14½ miles east of Kingston, 14 January 1947. The bird was a male, and in a letter dated 11 March 1947 he says the mites were 'extracted from a "growth" after macerating it for a while in caustic potash. This is the only "growth" of its kind I have found among approximately two hundred birds examined in Jamaica. The bird seemed quite healthy.' I have to thank Mr Thompson for his kindness in handing me this, together with other acarine material, for study and determination.

Hitherto some six species of the genus *Cnemidocoptes* have been known, all of them producing scab-like formations on the bills and legs of various birds. The best known species are *C. mutans* (Robin, 1860) and *C. gallinae* (Railliet, 1887), which produce the so-called 'scaly-leg' of chickens. A short but fairly detailed account of the former of these species was given by Megnin (1892). The new species now described is undoubtedly very nearly related to *C. fossor* (Ehlers, 1873), which was taken from the beak of *Munia maja*, the Maja finch, one of the Malaccan weaver birds; it differs considerably, however, in many important characteristics from this species and there can be no doubt of its true specific status.

Cnemidocoptes jamaicensis n.sp.

Female. Size very variable, length 300–360 μ, breadth 245–280 μ. The outline of the body is circular, as in *Cnemidocoptes fossor*, with which species it also agrees in having the dorsal shield longer than broad and having all the tarsi possessed of four claws. Posterior to the dorsal shield the chitinization shows a broad reticulate pattern occupying the whole of the central area of the dorsum. Around the borders of this area the reticulations are compressed, and the skin around this central area is finely striate (Fig. 1). The ventral surface is shown in Fig. 2. The formation of the epimera is clearly seen in this figure and is characteristic of the species. The female genital aperture is set very far back between the fourth legs and not as, for example, in *C. mutans*, between the second and third legs. Between the second and third pairs of legs, on the borders of the body, is a comparatively large and well-marked spine (Fig. 3) which has its posterior edge produced into a secondary, smaller spine-like process, more apparent in some specimens than in others. The large posterior hairs are much shorter in this species than in *C. mutans* and most other species of the genus, but they are longer than the posterior pair of *C. fossor*. The tarsus has four claws arranged as in Fig. 4, with three medium-sized upper ones and a somewhat larger lower claw. The mouthparts are flanked by the usual processes found in other species of the genus which are half twisted and produced into long fine points. The chelae have three cusp-like teeth on each digit, and on the movable digit only a well-developed pollici-form process set well back in the angle of the digits themselves. This appears to fit into a notch and an adjacent similar process, less well developed, on the fixed digit, and the whole suggests a locking device which, so far as I am aware, has not been recorded before for this genus. The palpi and the outline of their articulatory surface can be made out in Fig. 5; they do not project any noticeable distance beyond the lateral processes of the rostrum, and the distal segment of each is armed with what appears to be very minute and somewhat spine-like tuberculations, but these are very difficult to make out accurately. The palpi themselves seem to be devoid of hairs of any kind, and in this character they differ from *C. mutans*, in which species at least two outstanding setae can be made out on each palp.

All the dorsal setae are exceedingly minute and such as can be seen are shown in Fig. 1.

Larva. 140–150 μ long, 110–115 μ broad. This differs very little from the larvae of other species of the genus. The posterior area of the dorsum is coarsely and longitudinally striate with a dorsal shield of similar proportions to that of the female, but the lateral edges are perfectly straight. All the dorsal hairs are subequal in length and very much longer and more conspicuous than those of the female. The sternum is very much reduced, the epimera of the first pair of legs very nearly meeting in the middle line as do those of the second pair, although a little less

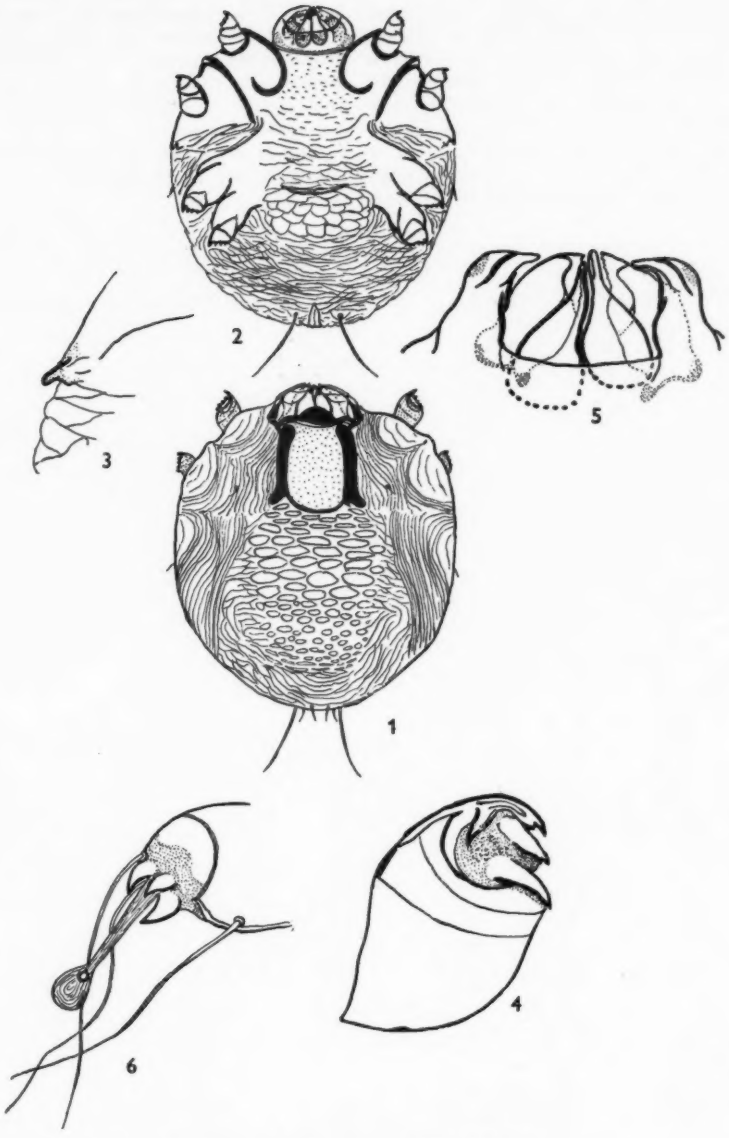

Fig. 1. *Cnemidocoptes jamaicensis* n.sp. Dorsum of female.
Fig. 2. *Cnemidocoptes jamaicensis*. Female, ventral.
Fig. 3. *Cnemidocoptes jamaicensis*. Female, lateral spine behind the second pair of legs.
Fig. 4. *Cnemidocoptes jamaicensis*. Female, tarsus of the second pair of legs.
Fig. 5. *Cnemidocoptes jamaicensis*. Rostrum and mouth parts of female.
Fig. 6. *Cnemidocoptes jamaicensis*. Larva, tarsus of the second pair of legs.

markedly so. This latter character is sufficient to differentiate this stage of the life history from the corresponding stage in other species of the genus. The posterior hairs are two in number and are exactly equal to the length of the body, and before birth are folded forwards beneath the body itself but seemingly are carried in the normal position immediately after birth. The first and second tarsi of this species are likely to be of diagnostic value and are figured in Fig. 6. Three claws are arranged on the dorsal edge of the extremity, and in the place of the fourth claw of the adult female is the usual tarsal 'sucker' mounted on a well-developed pedicel such as is found in the larvae of all the species of this genus for which such a form has been described. There is no reason to doubt but that the fourth claw of the adult is the homologue of the pedunculate sucker of the larva, and that it is the other claws which are most possibly secondarily acquired.

The syntypes are divided between my own and Mr Thompson's collection.

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STUDIES OF ACARI

VI. PARASITIC MITES FROM MAMMALIAN HOSTS OBTAINED IN CEYLON

By F. A. TURK, Ph.D., F.R.E.S., F.Z.S.

(With 15 Figures in the Text)

The present paper is mainly concerned with the records and descriptions of new species of parasitic acari obtained in Ceylon and sent to me during 1947 by Mr Gordon B. Thompson, of whose collection they form a part. The study of the specimens of the tropical rat mite, *Echinolaelaps echidninus* (Berl.), represented in this material led me to re-examine both the literature and the specimens of this species contained in my own collection, and I have included in the present paper the results of this investigation and have established various forms of the species which this necessitates besides showing the probable relationships existing between them.

Laelaps atypicus n.sp.

Type material consists of fourteen females, the syntypes. Measurements of type material: length, 600–800 μ, exclusive of the gnathosoma; greatest breadth 530–560 μ, the length being more variable than the breadth. The shape, for the most part, is subdiscoidal but very strongly shouldered and acuminate anteriorly. The dorsal shield (Fig. 1) covers almost the whole of the dorsum and leaves only a very narrow uncovered margin posteriorly. The setae on the dorsal shield to the level of the anterior margin of the second pair of legs are shorter than the remainder of the dorsal setae which, with the exception of the two median posterior pairs, are all subequal. There are no conspicuous shoulder bristles. The first two pairs of legs are markedly thicker than the hinder pairs when seen from above.

The sternal shield is markedly broader than long, and each of the three pairs of sternal hairs is set on a single small faint reticulation of its own. On the posterior border is a shallow, median concavity. The metasternal shields are well separated from the sternal shield, and each carries a seta which is a little shorter and thinner than those on the sternal shield itself. The genital portion of the genito-ventral shield is hyaline and trapezoid in shape. The ventral shield carries four pairs of setae situated very much as in other members of the genus. This shield is extremely broad posteriorly, its greatest breadth being about equal to the length of the shield which is produced posteriorly, so that it is nearly contiguous with the anal shield. The anal shield is triangular, its anterior border being rounded, and it carries the usual three circum-anal hairs of which the hinder unpaired hair is much the longest and strongest. Three rows of weak setae occupy the space between the ventral and anal shields and the posterior margin of the body. The peritremes are somewhat sinuate and are broader by the stigmata themselves and are, moreover, produced a considerable distance behind the stigmata and end in an acute point. By the fourth pair of legs and posterior to the metasternal shields are a pair of oblique sclerites, more apparent in some specimens than in others but present in all of them. These sclerites are of very doubtful homologies; I was inclined at first to think that they were detached portions of the endopodal shields, but further investigation showed that, although these latter were always very weakly chitinized in this species and extremely narrow, they are, nevertheless, always present. A possible origin of these oblique sclerites may be found by comparing this species with *Laelaps sculpturatus* Vitzthum, 1926, a form to which it does in fact show some similarities; in this species the metasternal shields are enormously developed—more so probably than in any other species of the genus—and they extend far behind the posterior border of the sternal shield. The metasternal hairs too are not placed at the posterior extremity of these shields but some distance back, leaving each shield very much produced beyond the hair. As the metasternal shields of *L. atypicus* are very much smaller it may be that the oblique sclerites are disassociated pieces of the metasternal shields. In some of the specimens it seems possible to make out a pair of very minute and weakly chitinized metapodial shields as well, although other specimens show no trace of these.

The first pair of legs are enormously developed, and their coxae are more than twice as large as the coxae of any of the other legs. The first and second pairs of legs are broader than the third and fourth pairs. The coxae of the first, second and third pairs of legs have each one spine only, and that of the fourth pair a single weak seta. This combination of characters appears to be unique in the genus *Laelaps* as known at present. The spines are of varying length and thickness and are best made out in Fig. 2. The

abnormal development of the genu of the first pair of legs is another atypical character.

Neither the hypostome nor the palpi show any very characteristic features, but the mandibles are curiously twisted, as may be seen in Fig. 3, which is drawn from a tangential view to show the peculiar correspondence of the teeth on the two digits, the two apical ones of the fixed digit fitting into the two peg-like and more proximal ones of the movable digit. However, unlike the condition of that genus the ventral shield of *Laelaps atypicus* does not surround the anal shield along its posterior border, although it is incurved in a very slight degree. It agrees, however, with other species of the genus *Laelaps* in having four pairs of setae on the genito-ventral shield as well as in lacking any of the bristles found at the base of the chelae in some other genera of the family. As in that genus too, the metapodial shields are

1

Fig. 1. *Laelaps atypicus* n.sp. Female dorsal.

The apex of the fixed digit is recurved and twisted, so that it lies in a plane tangential to the plane of its shaft.

It will be seen that this species does, indeed, show many features which are not typical of the genus, and in some respects it is somewhat similar to the characters found in the genus *Echinolaelaps*, especially in the enormous development of the ventral shield which, as in that genus, reaches to the anal shield. minute or absent. In some respects it shows a relationship to *L. haploti* Hirst, 1931, the female of which is figured by Wommersley (1937). Like the present species that species has the ventral shield developed posteriorly and its posterior border slightly incurved to fit the anterior border of the anal shield, and the two species agree also in having a single tooth-like spine on the coxae of the first and third pairs of legs as well as much the same differentiated

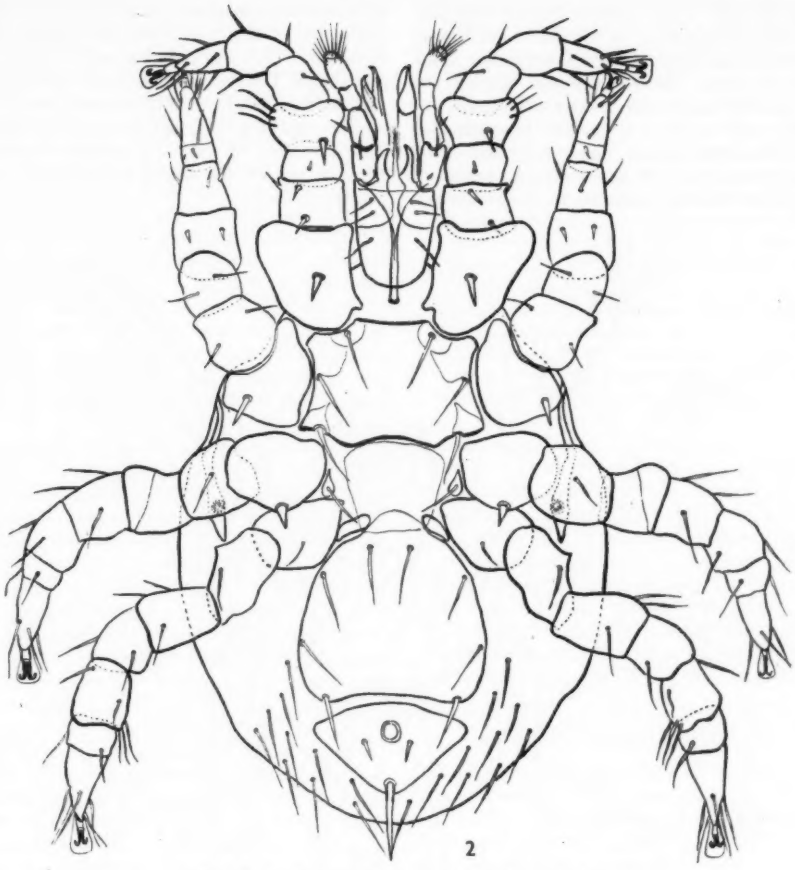


Fig. 2. *Laelaps atypicus* n.sp. Female ventral.

Fig. 3. *Laelaps atypicus* n.sp. Chela of female.

areas on the sternal shield. *L. haploti* was taken from the pouched mouse *Notomys*. The present specimens were taken from *Coelomys bicolor* Thomas, the bicoloured spiny rat, at Mousekande, Gammaduwa by W. W. A. Phillips on 27 June 1935 at an altitude of 3400 ft.

Macrolaelaps taprobanius n.sp.

Type material consists of two females, the syntypes. Measurements of type material: length, excluding the gnathosoma, 680 μ; breadth 410–415 μ. The general body shape of this distinct species is subrectangular, the sides being almost exactly parallel to each other. The dorsal shield covers nearly all the dorsum, leaving only a comparatively narrow area of the posterior of the body uncovered. The dorsal shield itself appears to be entirely unsculptured.

On the ventral side (Fig. 4) the sternum has a very characteristic shape and appearance, having a central portion projecting from the middle of the

anterior border and the posterior border strongly incurved. The reticulations on the shield are coarse and incomplete, and the shield itself carries the usual three pairs of hairs. The metasternal shields are somewhat pear-shaped and moderately well developed, and each carries a seta which is subsimilar to those on the sternal shield. The genital portion of the genito-ventral shield is hyaline and so demarcated from the ventral portion as to be almost

setae extend towards the sides of the body from about the level of the very thin semi-lunar metapodial scutulae, which can only be made out in one of the specimens. The peritreme is somewhat sinuate in the portion from the level of the second pair of legs backwards to the stigmata, and it is of equal width throughout. The area of the body surface immediately in front of the sternal shield is very finely and indistinctly striate.

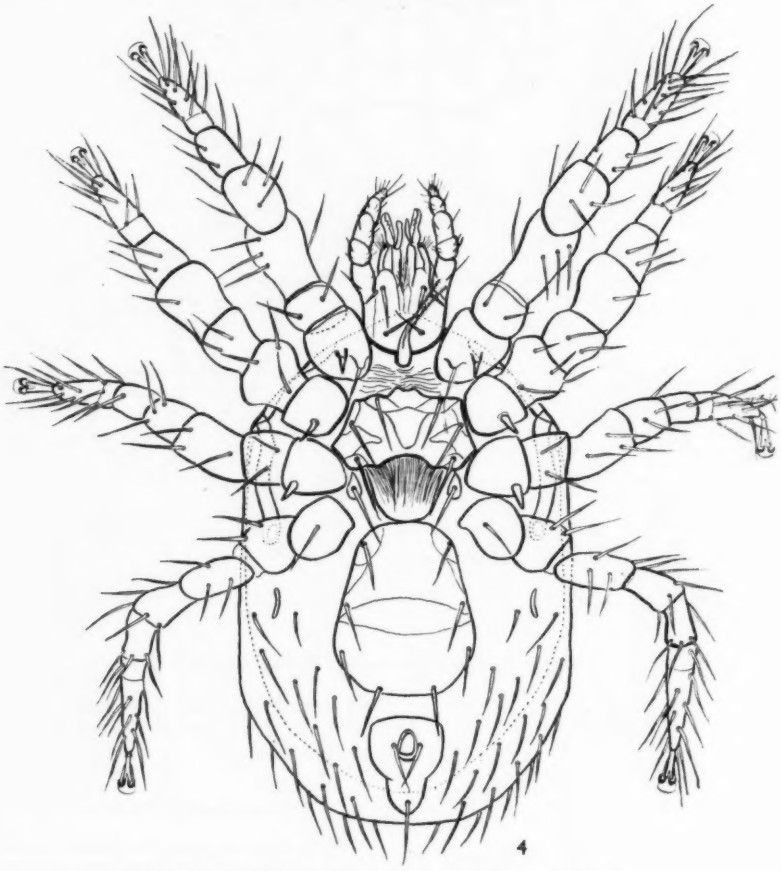


Fig. 4. *Macrolaelaps taprobanius* n.sp. Female, ventral.

separated, but as the chitinization is weak in this region it is difficult to make out the exact details of its structure. The ventral shield itself carries four pairs of hairs, an important character, for the significance of which see the remarks and comparison with *Macrolaelaps bhutanensis* Turk, 1946, which follow this description. The anal shield has a broad, bluntly ended, posterior projection and carries a long posterior post-anal seta. Four rows of long slender

The second pair of legs are the broadest and the first and third are subequal in thickness, whilst the fourth pair are the thinnest. The coxae of the first pair each bear a stout spine and a slender seta which latter is set on a well-marked tubercle. The coxae of the second and third pairs of legs have each a single spine, of which those on the third are somewhat the stoutest, whilst the coxae of the fourth pair of legs have each a single long slender seta only.

The chelicerae are large with very expanded shafts, and the stout movable digit bears at its base a small brush of flexible setae, rather few in number. Of the palpal segments the first and the third are the longest, whilst the apical one is remarkably small and is armed with only a few sensory setae.

There can be no doubt at all that this species is very nearly related to and certainly congeneric with *M. bhutanensis* Turk, 1946 which was collected from *Vandeleuria nilagirica nilagirica* Hasimara in Bhutan. Most of the remarks on the position of these forms which were appended to the description of *Macrolaelaps bhutanensis* (see Turk, 1946) must apply with even greater force to *M. taprobanius*. The two species agree very closely in the spinulation of the coxae of all the legs, the shape of the anal shield, the faintly striate area in front of the sternal shield, the comparatively small size compared with all the other species of the genus and, perhaps, the strangely similar reticulate pattern on the ventral shield. Of the generic identity of these two species there can be no doubt, although they differ in the formation of the peritremes which are sinuate in *M. taprobanius* and straight in *M. bhutanensis*, the much smaller and less developed metapodial plates in the former species, the shape of the sternum which is without any anterior projection in *M. bhutanensis*, and lastly that *M. taprobanius* has four pairs of setae on the ventral shield whilst the type specimen of *M. bhutanensis* has only three pairs. This divergence in what is usually a stable generic character in the Laelaptidae is at once explicable when the two species are compared. There can be little doubt but that the third pair of setae on the ventral shield of *M. taprobanius* are the homologues of the pair of setae that lie close to but just off the posterior angles of the shield in *M. bhutanensis*, where the chitinization of this area in the type of that species was neither so strong nor so extensive as in the former.

As more and more of these forms are discovered it becomes increasingly difficult to demarcate the limits of the genus *Macrolaelaps* and nearly related genera, especially *Gigantolaelaps* Fonseca (1939) and *Geneiadolaelaps* Ewing (1929). The last of these three genera was distinguished by Ewing chiefly by the unsculptured dorsum, but in the present species, *Macrolaelaps taprobanius*, we have a form seemingly belonging to *Macrolaelaps* yet without dorsal sculpturing. Previously, too, the very large size of the two former of the three genera listed above was thought to distinguish them from the other genus, but in *M. taprobanius* and *M. bhutanensis* we are discovering an oriental complex of species which are only half the size of the other known species of *Macrolaelaps*. Provisionally it would seem best to separate these three genera, all of which agree in having a brush of setae at the base of the digitus mobilis of the chelicerae, as follows:

Without stouter spines on any of the coxae. With three pairs of setae on the ventral shield. **Geneladolaelaps** Ewing, 1929

With stouter spine-like or peg-like setae on the coxae; always with two pairs of such setae on the coxae of the first pair of legs and one or two setae on all the other coxae. 2

2. Genito-ventral plate expanded behind the coxae of the fourth pair of legs and with a variable number of setae but always more than one pair. Lateral posterior ventral setae never more than five rows and never reaching to the fourth coxae. **Macrolaelaps** Ewing, 1929

Genito-ventral plate not, or only a little, expanded behind the coxae of the fourth pair of legs and always with only one pair of setae. Lateral, posterior, ventral setae always more than five rows and reaching or nearly reaching to the fourth coxae. **Gigantolaelaps** Fonseca, 1939

It may eventually be found proper to separate *Macrolaelaps bhutanensis* and *M. taprobanius* in a subgenus of their own which will probably be found to include the Indian forms. Such a subgenus would be characterized by the smaller size, the somewhat elongate anal shield, and by the fact that the outer of the two spines on the coxae of the first pair of legs is set on a tubercle.

The type material of *M. taprobanius* was collected on *Bandicota malabarica* at Gammaduwa, Mousekande, Ceylon, by W. W. A. Phillips, 31 May 1948.

Haemolaelaps nuttalli Hirst, 1915

Two females of this species were taken on *Rattus rattus kandyianus* at Gammaduwa, Mousekande, Ceylon, on 13 April 1935 by W. W. A. Phillips. These specimens were 610 and 630 μ long respectively and 410 μ broad at the greatest breadth. The measurements of the dorsal shield are rather more variable, being 580 by 330 μ and 560 by 360 μ in each specimen. Hirst (1915) gives the measurement of the type specimen as 620 and 440 μ.

Haemolaelaps thompsoni n.sp.

This species greatly resembles the previous one, but is easily distinguished by its larger size. Seven females of this species were taken, the syntypes; their lengths (excluding the gnathosoma), vary between 930 and 1050 μ, and the greatest breadth between 660 and 750 μ. The body is broadly oval in outline, and the dorsal shield leaves a considerable amount of the body uncovered at the margins, these uncovered margins extending far forwards. The dorsal shield is rather densely covered with long and somewhat flexible setae and its surface is reticulate, the reticulations being compressed in an antero-posterior direction. The vertical hairs are shorter and

stiffer than the other dorsal setae. The dorsal shield itself is rounded posteriorly and well-shouldered anteriorly.

On the ventral surface (Fig. 5) the sternal shield has a characteristic configuration; the anterior margin is sinuate and produced forwards at the sides, the shield itself carrying the usual three pairs of hairs. The metasternal shields are placed obliquely and shaped as the arms of a right angle, the basal arm being wider than the other. These shields touch the sternal

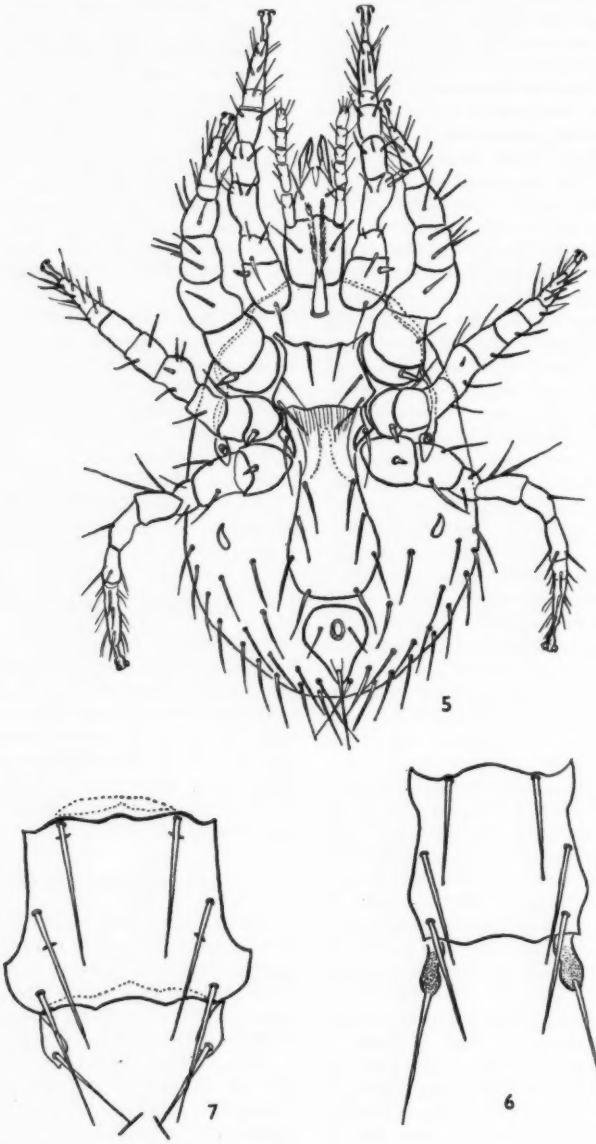


Fig. 5. *Haemolaelaps thompsoni* n.sp. Female ventral.
Fig. 6. *Echinolaelaps hirsti* n.nom. Sternum of female. Copied from Hirst (1913).
Fig. 7. *Echinolaelaps echidninus* subsp. *vitzthumi* n.subsp. Sternum of female. Copied from Vitzthum (1926).

shield and are more highly chitinized in those individuals which have all the other shields more weakly chitinized. The genito-ventral shield is vase-shaped, more abruptly truncate posteriorly than in *Haemolaelaps nuttalli*. The anal shield is longer than broad and more acuminate posteriorly than in the latter species, and the post-anal hair is at least three times as long as the other anal hairs, whereas in Hirst's figure of his species this hair is only about twice as long as the others. Metapodial shields are present, somewhat comma-shaped. The peritremes are sinuate and produced beyond the stigmata.

The second pair of legs are thicker than the others, and the fourth pair carry very long setae on the dorsal surface of the femur and genu, a marked characteristic of this species. A long seta and a short peg-like spine are present on the coxae of the first pair of legs, and a single peg-like spine is present on each of the other coxae, those on the second pair of legs being the largest and those on the fourth pair the most reduced, but even so they are stouter than those figured by Hirst on the coxae of the fourth pair of legs in his species.

All the specimens were taken from *Rattus rattus kandyianus* at Gammaduwa, Mousekande, Ceylon. Females were collected on 28 January and 4 July 1935.

Echinolaelaps echidninus (Berlese, 1887)

This is the common rat mite which has been recorded many times from widely separated localities in the tropics and from docks and similar areas in other parts of the world. Considerable importance attaches to this species, as it is the intermediate host and vector of *Leucocytogregarina muris*. Berlese (1912) records also an '*Hepatozoon perniciosum*', which is the causative agent of a fatal epidemic leucocytosis in rats and related rodents, and the mite has been suspected of playing a possibly similar role in the spread of other infections.

Seemingly, however, a variety of rather widely different forms have been recorded under the same name without any attempt having been previously made to distinguish between them. Ferris (1932) says: 'Apparently it has been the custom to refer all specimens of *Laelaps* having the posterior border of the genito-ventral shield excavated to fit the anterior border of the anal plate to *L. echidninus*.' In the present collection of material from Ceylon I have specimens taken from *Rattus rattus kandyianus* and from *Vandeleuria rubida* Thomas, and the comparative study of these has led me to review the main literature of the species.

The species was first described very briefly by Berlese in 1887 when only the female was known, but a good and detailed figure was published by Berlese in 1912. Hirst (1913, 1914*a*) subsequently described and figured a protonymph, deutonymph, male and female, of what he took to be this. Vitzthum (1926) recorded, described and figured specimens from Java which he referred to this species. Wommersley (1937) recorded the species from Australia, where, besides its usual occurrence on *Rattus rattus* and *R. norvegicus*, it was collected from *R. culmorum* and *Perameles gunni*. No description or figure was given of any of these specimens but they were presumably females, and as they included some previously identified by Hirst they were, presumably, described and figured by that author. Trägårdh (1931) described and figured a *Laelaps pallidus* which, as he pointed out, is very closely related to *Echinolaelaps echidninus*, from Juan Fernandez island, and there can be no doubt but that this is an *Echinolaelaps* sp. Ferris (1932) doubted whether Trägårdh's material constituted a new species, but in spite of the similarity which his description and figures show to those of Hirst, there appears to be little doubt, from the characteristic of the metasternal shields, the dorsal shield and the large pores in the sternal shield, that this form is a subspecies at least. Although there are many other records of this species from various parts of the world, these references seem to constitute the main literature of the species with the exception of the criticism of Berlese's description contained in a paper by the late Dr A. C. Oudemans (1928). Buitendijk (1945), in her catalogue of the Oudemans' collection, lists specimens of *E. echidninus* from the Dutch East Indies, the Malay Archipelago and Malacca, and it would seem most probable that these would at least contain forms similar to those figured by Vitzthum. As Trägårdh has pointed out, however, Oudemans seems to have misunderstood what Berlese intended, and Trägårdh has shown, moreover, that it is probable that Oudemans had overlooked the drawing given by Vitzthum.

One of the most obvious differences to be seen when comparing these descriptions and figures, and, indeed, any series of specimens of this mite, is the degree of development of the genito-ventral shield and the shape of its outline. Although no previous author seems to have called attention to this fact, my material shows that this character varies very considerably from specimen to specimen, and in Fig. 9 I have superimposed the outlines of one side of the ventral portion of the shield as it is seen in four specimens of this species all taken from one individual of *Rattus rattus kandyianus*, all these mites being adult females. The wide range of variation will be apparent at once, and as these are all from the same species of mite from the same host individual, it seems obvious that this character is worthless for the purpose of separating the different forms.

In Figs. 6–8 I have copied the sterna of the female from the figures given by Hirst, Vitzthum and

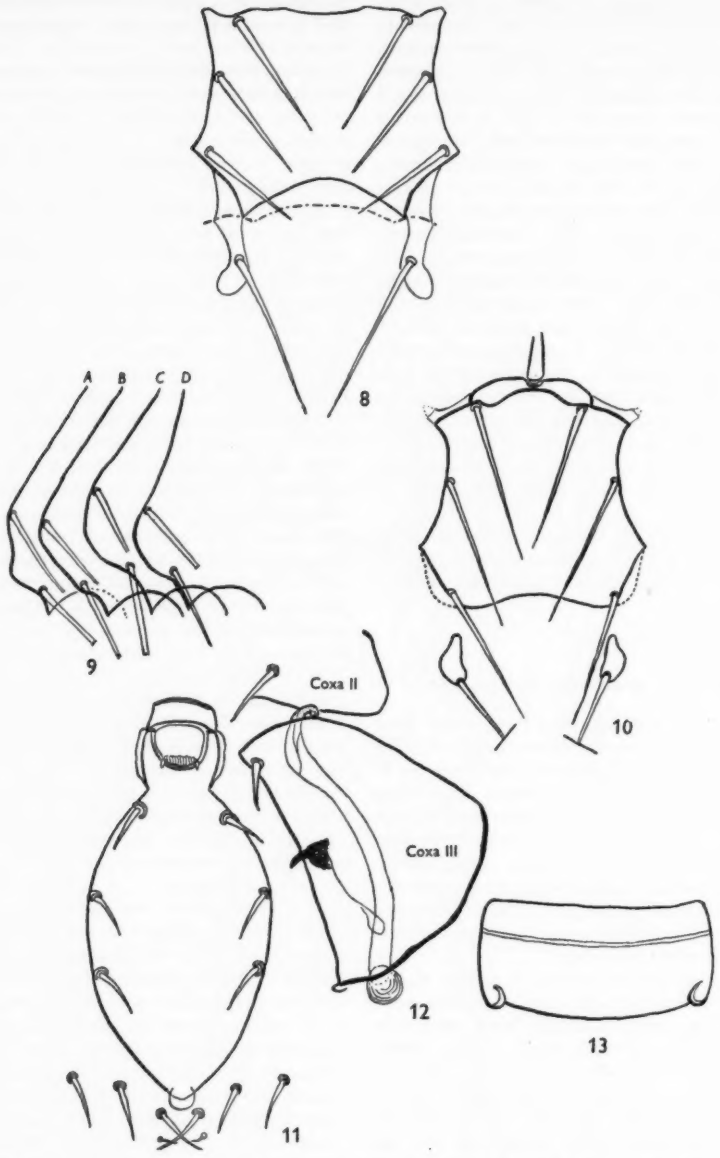


Fig. 8. *Echinolaelaps echidninus* Berl. 1887. Sternum of female. Copied from Berlese (1912). The dotted line has been added to show the supposed line of fusion of part of the metasternal shields with the sternum.
Fig. 9. *Echinolaelaps hirsti* subsp. *ceylonicus* n.subsp. Outlines of the ventral shields of four females to show the degree of variation.
Fig. 10. *Echinolaelaps hirsti* subsp. *ceylonicus* n.subsp. Sternum of female.
Fig. 11. *Spinturnix orientalis* n.sp. Male; jugular and sternal shields.
Fig. 12. *Spinturnix orientalis* n.sp. Male; the peritreme, showing its relation to coxae 3 and 4.
Fig. 13. *Meristaspis lateralis* subsp. *ceylonicus* n.subsp. Jugular shield of female.

Berlese respectively, and in Fig. 10 I have drawn the sternum of a specimen taken from *R. rattus kandyianus*, which is the same individual as the one having the outline of the ventral shield as in Fig. 9C. Comparison of these figures shows some astonishing differences, and they may be at once classified into two main types, viz. those in which the greatest width is more than the greatest length (Berlese's and Vitzthum's) and those in which the length is greater, or equal to, the breadth (Hirst's and the specimen drawn in Fig. 10). Comparing Berlese's figure with that of Vitzthum it will be seen that the differences are scarcely less great than those between the two main types as shown in Berlese's and Hirst's drawings. Vitzthum shows areas of variable chitinization along the anterior and posterior border of the sternum which are not figured by Berlese. In Berlese's figure the metasternal shields are very large and fused with the sternum proper, and the third pair of sternal hairs are placed very far forwards. In that of Vitzthum the metasternal shields are small and separated, and the third pair of sternal hairs are very near to the posterior border of the sternum. There are other minor differences apart from those to be found in the sternum; the vertical hairs shown by Berlese are twice as long as those of Vitzthum's specimens, and Berlese shows the coxal spines, especially those on the third and fourth legs, as being very much stouter than in Vitzthum's figure.

The length of the female as given by the two authors shows a great discrepancy also; Berlese gives 800 μ and Vitzthum 1040–1240 μ. Lastly, the metapodial shields shown by Berlese are three time as large as those figured by Vitzthum. These differences would seem to justify us in treating the form dealt with by the latter as a subspecies, and I propose the name *Echinolaelaps echidninus* subsp. *vitzthumi* n.subsp. for this purpose.

The form attributed to the species under discussion by Hirst differs in a great many respects from that of Berlese, as will be apparent when the figures of the two authors are compared. The difference in the relative sizes also is as striking as that instanced between Vitzthum's and Berlese's forms, for Hirst gives the total length of the females which he recorded as 1100 μ, 300 μ more than Berlese's. As Vitzthum's drawing and description is more detailed than that of Berlese, and as it may be safely assumed that the two are conspecific, the differences between them being no more than subspecific ones, it will better serve the purposes of comparison if the differences found to exist in the drawings and data of Vitzthum and Hirst are enumerated. Hirst's figure shows the greatest width of the sternum (by the anterior border of the coxae of the third pair of legs) to be almost exactly equal to its greatest length; the sternum drawn by Vitzthum shows the width to be one and one-third times the length. The anterior of the sternum of Hirst's form is produced laterally and anteriorly, and the median projection of the anterior border is far more marked than in Vitzthum's form. The conformation of the posterior third of the sternum in each instance is completely different, as will be seen by comparing Figs. 6 and 7. The metasternal shields of Hirst's form are fused with the sternum, whereas Vitzthum shows them as being only just contiguous. In addition to these differences Hirst's form has the metapodial shields elliptical and the anal shield longer and more slender and set fairly far forwards from the posterior margin of the body. In Vitzthum's figure the metapodial shields are rounded and the anal shield is much broader and reaches nearly to the posterior margin of the body. The last-mentioned character should not, I think, have too much emphasis laid upon it. I possess specimens of this species taken from *Vandeleuria rubida* in Ceylon which agree almost exactly with the description and figures given by Hirst but the anal shield of my specimens is broader than long (as 860 to 800), whereas Hirst's figure shows it to be longer than broad (as 875 to 1000); in each case the length measurements have been made to the posterior edge of the insertion of the post-anal hair. The similarity of these specimens from *V. rubida* with the female figured by Hirst is otherwise almost complete, the only other difference that I can find is that the sternum is a little more obviously produced between the third and fourth pairs of legs. The chelae of these specimens agree exactly with the description given by Hirst. There remains one other characteristic to which attention should be called: Trägårdh (1931) has discussed the distribution of the peculiar areas of thin depressed cuticle which are present at the anterior of the dorsal shield. As he points out, Vitzthum does not describe or delineate these although he does so for other species, whilst Berlese delineates three such areas. Hirst, too, does not mention them in his description of the female. In *Echinolaelaps pallidus* (Träg.), which is certainly very closely allied to the form which Hirst has dealt with, Trägårdh describes and delineates eight such areas (see Trägårdh, 1931, Fig. 145). The specimens mentioned above which were taken from *Vandeleuria rubida* show six such areas which are placed exactly as the anterior six shown by Trägårdh, with the exception that the two anterior lateral ones are larger than in *Echinolaelaps pallidus* and the median posterior one is very well developed, of an elongate wedge-shape with the thin edge of the wedge pointing forwards. In my specimens, too, none of the areas are subdivided into smaller areas as in *E. pallidus*. This not only serves to emphasize the very close relationship between *E. pallidus* (Träg.) and Hirst's form, but it adds yet one more reason to those already discussed in this paragraph, for considering Hirst's form to be a separate species. For this species I propose

the name *E. hirsti* syn. *Laelaps echidninus* Hirst, 1913 non Berlese, 1887. The lectosyntypes will be the specimens taken from rats captured at Tilbury docks by Dr Willoughby, referred to by Hirst in his paper of 1913 and now in the British Museum (Natural History). Trägårdh's form will best be treated as a subspecies of *Echinolaelaps hirsti* and should be designated *E. hirsti* subsp. *pallidus* (Träg. 1931).

The specimens of this mite which were contained in the present collection and which were taken from *Rattus rattus kandyianus* captured at Mousekande, Ceylon, present a further problem. There can be no doubt that they are referable to *Echinolaelaps hirsti* as will be seen from the figure of the sternum (Fig. 10), which is certainly of the type of Hirst's form. There are, however, differences of fairly considerable magnitude which, it is interesting to note, affect the deutonymph as well as the adult and give reason for presuming that all the subspecies are differentiated in the immature stages as well as in the adult, although there is not yet enough material collected to allow of our being certain of this. The differences which exist between the specimens at present under discussion and those of Hirst may be set out shortly as follows: The deutonymph differs from Hirst's species in that the posterior end of the sternum is much narrower and more prolonged. The peritreme is straight and not sinuate, and its internal posterior border is toothed. Hirst (1914*a*) does not describe or figure the legs of the deutonymph of his form, and possibly one may conclude that there was no outstanding character to be noticed on these appendages; the specimens which I have from Ceylon, however, have enormously developed clavate spines on the femur, genu and tarsus of the second pair of legs and so conspicuous is this feature that I believe that it cannot be present in the forms examined by other authors. No description of the chelicerae of the deutonymph seems to exist, but my specimens have four teeth on the fixed digit, all of which are recurved, whilst the apical part of the digit is itself incurved. This is certainly very different to the condition of the same structure found in the female. The female differs from the form figured by Hirst in that the anterior border of the sternal shield is not turned forward as in the type but is straight, and the posterior lateral borders are rounded and not incurved although the chitinization of this area is weak and not exactly the same in each specimen. The metasternal shields are completely separate from the sternal (Fig. 10), and the metasternal hairs have their origin on the posterior extremity of the shields. It would seem that the place of insertion of these hairs may be important, as the condition in these specimens agrees exactly in this respect with that figured by Hirst (Fig. 6), and is in both instances different to the condition of this character figured by Berlese and Vitzthum; moreover, I find no individual variation in the specimens which I have examined. The metapodial shields of these specimens are more nearly triangular than that delineated by Hirst, and, as has already been pointed out, the variations in the ventral shield do not provide a reliable character for comparison to be made between the two forms. For all these specimens which were collected from *Rattus rattus kandyianus* at Gammaduwa, Mousekande, Ceylon, I propose to erect a new subspecies of *Echinolaelaps hirsti mihi* to be named *ceylonicus* n.subsp., of which these shall be the syntypes. To the above description of these should be added the dimensions of this form, viz.: length of female, 1080–1200 μ with breadth 730–830 μ; length of deutonymph, 610 μ, breadth 410 μ.

In this short study of these forms the following have been recognized: *Echinolaelaps echidninus typicus* (Berlese, 1887); *E. echidninus* subsp. *vitzthumi* n.subsp. = *E. echidninus* (Vitzthum, 1926) non Berlese, 1887; *E. hirsti* n.sp. = *E. echidninus* Hirst, 1913 and 1914 non Berlese, 1887; *E. hirsti* subsp. *ceylonicus* n.subsp.; *E. hirsti* subsp. *pallidus* (Trägårdh, 1931) = *Laelaps pallidus* Träg. 1931. There is a further *Echinolaelaps* species to be found described under the name *Laelaps rothschildi* Hirst 1914; this form was taken from *Uromys stalkeri calidor* Thos., *U. lorentzi* Jentinck and *Mus* sp. in Dutch New Guinea. It is almost certainly a separate species related to but well differentiated from the subsp. *vitzhumi*. It is characterized by the very wide genito-ventral plate and the minute dorsal and ventral setae and thus should become *Echinolaelaps rothschildi* (Hirst, 1914*b*). Finally, there remains to be considered the relationships of these forms and the kind of systematic category which would best express this. The foregoing paragraphs have shown that the most reliable character for the separation of the two species and their related subspecies would appear to be found in the shape of the sternal shield and the degree of fusion which it shows with the metasternal shields as well as the relative positions of the point of origin of the sternal and metasternal hairs on their respective shields. The two species *Echinolaelaps echidninus* and *E. hirsti* seem to be well separated by the ratio of the length to the breadth of the sternal shield, and no intermediate forms are known of this character. The stages in the varying degrees of fusion of the metasternal with the sternal shield are apparently not so clear-cut. Provisionally, I would suggest that the variations in this character may best be explained by supposing that the metasternal shields of Berlese's form have partly fused with the sternum along the line indicated in Fig. 8, and that the remaining posterior portion has gradually drifted away backwards in the course of evolution, in the order *E. echidninus typicus* → *E. hirsti* → *E. echidninus vitzthumi* → *E. hirsti ceylonicus*, in which latter they are quite

separate as they are in *E. hirsti pallidus*. Correlated with this process, it would seem that, at the same time, the third pair of sternal hairs and the metasternal hairs have also passed posteriorly. In this last respect, however, *E. hirsti pallidus* seems to be somewhat aberrant, as in this form the hairs in question seem to have retained their primitive (?) position.

The interpretation of these relationships with the facts at present available must remain open for further investigation. However, such facts suggest that all these forms may be considered a supraspecies as this term is defined by Huxley (1940), i.e. a group in which, of the constituent groups, 'some have remained obviously subspecies while others have differentiated to a stage at which they are, or appear to be, full species'. It is probable that the forms of *E. echidninus* and *E. hirsti* each compose a 'Rassenkreis' in the sense in which Rensch used this term, but geographical replacement is unlikely to be completely exclusive when one considers the way in which the hosts of some forms (*Rattus* spp.) distribute themselves over the world.

Spinturnix acuminatus Koch, 1836

Three specimens, almost certainly of this species and all of them males, were taken from Wroughton's bat, *Scotophilus wroughtoni* Thomas, captured at Kokkalai near Palmoddai, Ceylon, by W. W. A. Phillips, 13 June 1936. This I believe is the first extra-European record for this mite, the usual hosts of which are *Nyctalus noctula* and *Pipistrellus pipistrellus*.

Spinturnix orientalis n.sp.

The single specimen of the male of this species, the holotype, was taken from *Taphozous* (*Saccolaimus*) *saccolaimus* Temminck, 1835, captured at Gammaduwa, Mousekande, 5 January 1935, by W. W. A. Phillips. The type is in my collection.

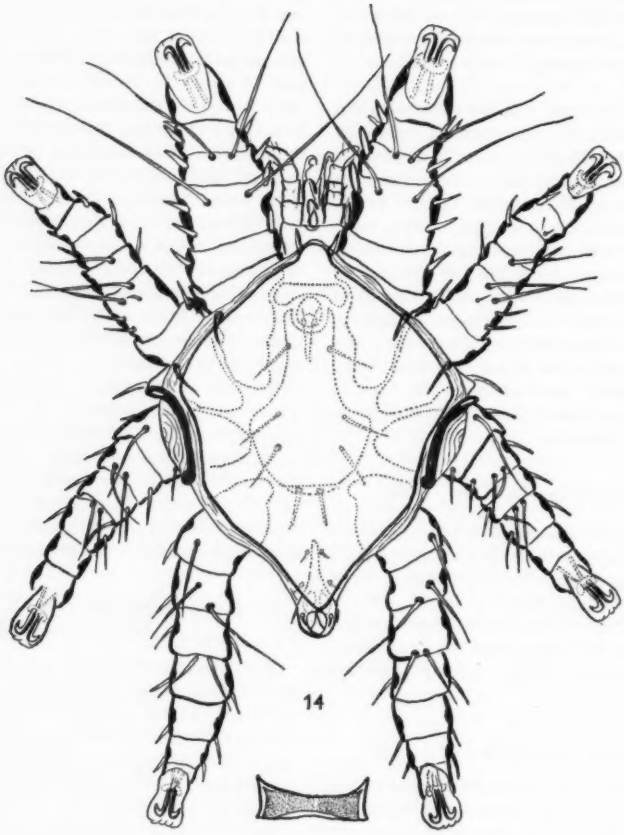
Although only the type specimen so far exists, it is sufficiently distinct from all other species of the genus to warrant it being given specific rank, and in view of the wide range of the host, viz. India, Ceylon, Burma, Malacca, Sumatra and Java, it will probably be proved to be widespread throughout much of the East. Male: 1080 μ long, 710 μ broad. The general facies are very similar indeed to *Spinturnix vespertilionis*, but detailed examination shows a great number of important differences. The dorsal shield is like that of the last-named species, but before the shield there seems to be only three pairs of hairs and not four as in that species. The new species has a jugular shield (Fig. 11) which is absent in *S. vespertilionis*; the sternal shield is highly characteristic and is best made out in Fig. 11, from which it will be seen to differ from all the known species of the genus. The sternal hairs are all very strong and exceptionally well developed and have their origin in extremely large follicles. A somewhat oblique row of six hairs lies behind the sternal shield, and posterior to the median pair of these are two longitudinal rows of similar hairs extending to the hind border; the first pair of these, however, are comparatively minute (Fig. 11). On the dorsal shield itself there appears to be no sculpturing, but this may be impossible to make out as the specimen is mounted in Canada balsam. Behind the dorsal shield are two pairs of short hairs. All the setae on the underside of the first pair of legs are very short; the longer lateral setae which are found in several other species of the genus are absent. On all the coxae is the usual median spine which is rather more developed in this species, those on the coxae of the first pair of legs being larger than the others but all of them tend to assume a somewhat incomplete L-shape. The peritreme is at once diagnostic of the species and is shown in Fig. 12. There are no sinuations in its whole length, as may be seen from the figure, and on the ventral surface of the animal only a very small portion of the whole of this shield extends into the space between the second and third coxae. This character alone seems to be sufficient to prevent the species being confused with any other species of the genus so far known.

Meristaspis lateralis Kolenati subsp. ceylonicus n.subsp.

Two females, the syntypes, of this new subspecies were taken from a female of *Rousettus seminudus* (Kelaart, 1850) captured in the Matale district, Rattota, Ceylon, by W. W. A. Phillips, 14 September 1935. This subspecies appears to differ from the typical form in the following respects: the two pairs of lateral, posterior hairs are equal (in the type the inner pair are smaller than the others). The median anterior setae on the posterior dorsal shield are much larger—nearly twice as long—as in the type. The posterior process on the coxae of the third pair of legs is not so well developed, and lastly, the shape of the jugular shield, which is well chitinized, is different to that found in the typical form. The jugular shield of the subspecies *ceylonicus* is shown in Fig. 13.

Meristaspis taprobanius n.sp.

Syntypes: one male and four females taken from *Rousettus seminudus* (Kelaart, 1850) captured by W. W. A. Phillips, 14 September 1935, in the Matale district, Rattota, Ceylon. All these specimens were taken from the same host and at the same time as the preceding subspecies. Of the species so far known in this genus, the present species is probably most nearly related to *Meristaspis lateralis* Kolenati, 1856,



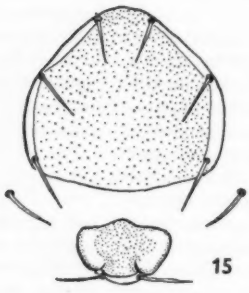


Fig. 14. *Meristaspis taprobanius* n.sp. Male dorsal.
Fig. 15. *Meristaspis taprobanius* n.sp. Female: jugular, sternal and genital shields.

but it is easily distinguished from that species by many characters as will be seen by comparing Figs. 14 and 15, and the following description with that given by Hirst (1923) in his redescription of Kolenati's species.

Female: 660–750 μ long, 410–460 μ broad. The dorsal shield is shorter than that of *M. lateralis* and not so sharply acuminate; it reaches only to the middle of the fourth coxae. It has a few sparse, short, spine-like hairs scattered rather irregularly over the surface. The transverse line which divides the dorsum into two parts seems to be in approximately the same position as in *M. lateralis*, but in the present species the dorsal shield does not reach back as far as this. The short hairs which lie on either side of the posterior end of the dorsal shield in *M. lateralis* appear to be entirely absent in *M. taprobanius*. The process on the dorsal surface of the capitulum is backwardly directed in both species. The jugular shield, which is very well chitinized, is of quite different conformation in the two species; in *M. taprobanius* the anterior border is very strongly incurved and the posterior border is straight, the whole shield being very much narrower than in *M. lateralis*. The sternal shield is about as wide as long and a very different shape to that which is assumed by this shield in the latter species, and the fourth pair of hairs (the metasternal setae) are not so far removed from the shield as in that species. The genito-ventral shield is of an extremely characteristic shape (Fig. 15). The hind border of the body is 'festooned' and has an anal protuberance. Neither the second nor the third pair of coxae carry the lobe-like posterior spur which is found in *M. lateralis*. All the legs carry some longer dorsal hairs on various segments, of which those on the first pair of legs are very slightly the longest and very much the stoutest, all being incurved.

Male: 580 μ long, 360 μ broad. The shape of the body and the dorsal shield is shown in Fig. 14. It is very similar to that of *M. lateralis* but is a little more acuminate posteriorly. There are three pairs of hairs in front of the dorsal shield. The legs are similarly proportioned to those of *M. lateralis*, but the longer dorsal setae of the first pair are very much longer than the corresponding hairs found in that species. The jugular plate is very narrow, being at least three times broader than long and is extremely strongly incurved on its ventral border. The sternal shield is extremely large and completely characteristic of the species as will be seen from Fig. 14, and the fourth pair of hairs is set on a well-demarcated portion of the shield at the posterior extremity which is marked off from the rest of the shield by a transverse line of thickened chitin; this portion undoubtedly represents the fused metasternal shields, and I know of no other species of the genus which show such a structure, although a somewhat similar chitinized portion is figured and described by Radford in his recently published (1947) descriptions of the males of *Ancystropus* (*Meristaspis*) *kenyaensis* and *A. jordani*. In these species, however, this portion of the shield carries four hairs in a linear transverse series across the posterior border of the scutum, and this extra pair is very difficult to account for unless, perhaps, they represent the fused inguinal shields which may have been possessed primitively by this group of mites. The ventri-anal shield is pointed anteriorly and of very characteristic shape, narrower than in any other known species of the genus. All the coxae have the usual small spiny process in the middle of the distal margin, and there is, too, one single hair near the inner angle of the coxae of the first and fourth pairs of legs and, as in *Meristaspis lateralis* this appears to be absent from the coxae of the second and third pairs of legs. This condition, I regard as a generic character of considerable importance, in that it occurs only in *Meristaspis* and *Ancystropus*, which Radford (1947) and some other authors regard as one genus, and is absent in other genera of the family. The two last-mentioned genera I regard as being quite distinct, since the absence of a pulvillus on tarsus 1 of *Ancystropus* is a character given great importance in other families of the Parasitoidea. Moreover, in the two genera under discussion the presence of the pulvillus seems to be correlated with a reduction in the size and, to some extent, the number of spines on the various segments of the first pair of legs.

The syntypes of each species, except where otherwise stated, are divided between the collections of Mr Gordon Thompson and myself.

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PARASITIC COPEPODS OF FISH FROM CEYLON. III

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(With 60 Figures in the Text)

Most of the nine species dealt with in this paper were collected before the outbreak of World War II. Library facilities in Ceylon, meagre enough even in normal times, were altogether unavailable during the war years owing to the removal of the Colombo Museum Library to safe areas inland. It was only after the library was brought back to Colombo at the beginning of 1947 that this paper could be got ready for publication. Hence it appears 10 years after the last contribution in the series.

Heegaard (1945) finds, in copepods, that the first maxillae have either entirely disappeared or are present as rudiments only, the appendages hitherto called the first maxillae are in reality the second maxillae and there are two pairs of maxillipeds. This view would indicate a return to the system of enumeration of copepod appendages that was in use at the end of the last century but has not been adopted here as it is not known whether it is generally accepted.

(1) **Parapetalus hirsutus** (Bassett-Smith)

Host and record. Three females and a male were obtained from the inner surface of the operculum of a *Polynemus tetradactylus*.

Remarks. This species, taken from the same host at Bombay, was originally described by Bassett-Smith (1898) as *Caligus hirsutus*. Wilson (1912) obtained two females, also from the same host, from Batavia, and transferred the species into the genus *Parapetalus* Steenstrup & Lütken. The inaccuracies in Bassett-Smith's observations have been commented on by Wilson, whose remarks concerning the shape of the cephalothorax and the number and arrangement of the setae on the fourth legs are in agreement with the conditions in the present specimens. The nature, however, of the setae on the fourth legs appears to differ from that described by both these authors. Bassett-Smith, apart from noting that the inner border of his 'first' seta (actually the second) is crenate, merely described the setae as 'short, thickened, slightly curved, simple'. Wilson described them as 'winged spines'. In point of fact, each seta is shaped like a shovel, the cylindrical base of attachment to the limb representing the handle of the shovel (Fig. 4). The rim of the shovel is crenate. These setae are suggestive of an additional modification for clinging to the host. Three groups of the unique, rosette-like 'hairs' described by Bassett-Smith are present.

A few other points are worth mentioning. The abdomen of the female is polygonal, not oblong as in the previously described specimens (Fig. 1). The genital segment of the male is flask-shaped (Fig. 2). The two-jointed character of the abdomen is not evident in the female, but in the male it is indicated by the presence of a narrow anterior portion and a wider posterior portion. The maxilliped of the male differs from that of the female, for while in the latter this appendage is of the usual form, in the former the tip of the claw is forked and apparently works against a group of three stout spines arising from near the base of the first joint (Fig. 3).

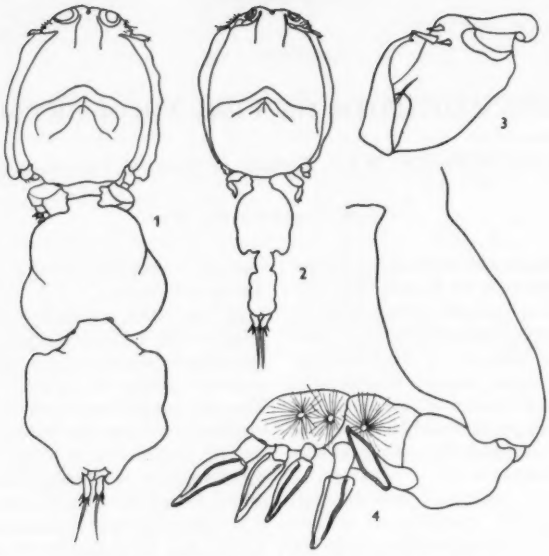
Length: female, 6 mm.; male, 4 mm.

(2) **Dissonus furcatus** n.sp.

Host and record. Several females, including juveniles, and males were found on the gill lamellae of an orectolobid shark.

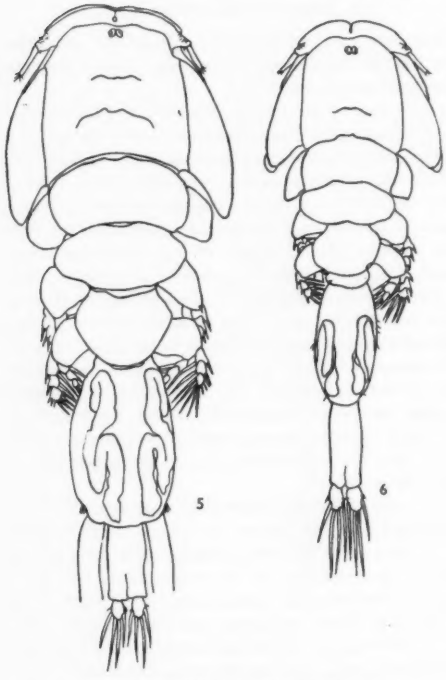
Description of the female (Fig. 5). Cephalothorax broader than long. Eyes situated close to the anterior margin, in contact with each other in the middle line. Second and third thoracic segments of the same width as the body of the cephalothorax. Lateral plates of the second thoracic segment not quite as wide as the lateral lobes of the cephalothorax. Fourth thoracic segment narrower but longer than the two preceding segments. Genital segment oval, much longer than broad, its ventral surface bearing bifurcated spines scattered in an arc almost confined to the anterior third of the segment. Abdomen of one joint, about two-thirds the length of the genital segment and much longer than broad. Anal laminae oval, each lamina bearing four large plumose setae and two much smaller setae, one on the inner and one on the outer margin.

First antennae two-jointed with numerous setae on the first joint and a few at the tip of the second. Second antennae also two-jointed, hook-like, armed with two short spines on the inner side of the hook. First maxillae arising on each side of the base of the mouth cone and extending well beyond it, each maxilla bearing on its ventral surface a short papilla with three setae, one of which is distinctly longer than the other two. At its tip each maxilla carries a long spine. Second maxillae long, two-jointed, the second joint terminating in two spines, the outer



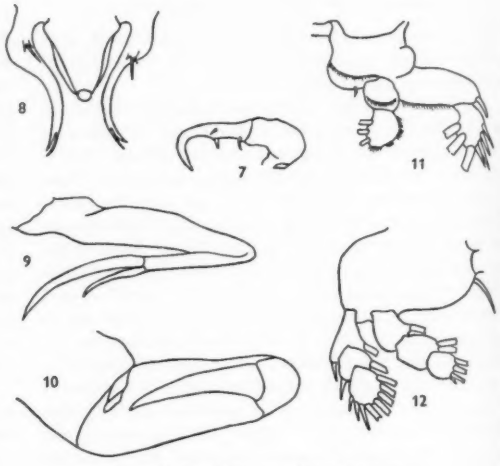
Figs. 1–4. *Parapetalus hirsutus* (Bassett-Smith).

Fig. 1. Female, dorsal view. Fig. 2. Male, dorsal view. Fig. 3. Maxilliped of male. Fig. 4. Fourth leg of female.



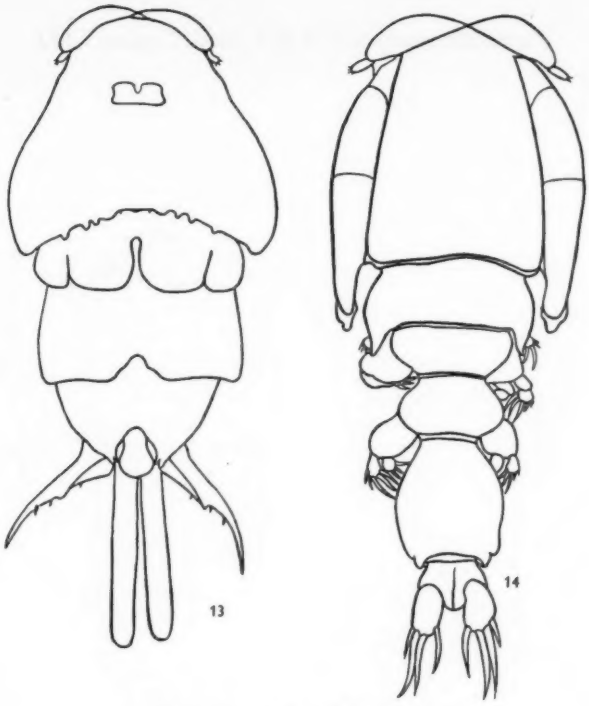
Figs. 5–6. *Dissonus furcatus* n.sp.

Fig. 5. Female, dorsal view. Fig. 6. Male, dorsal view.

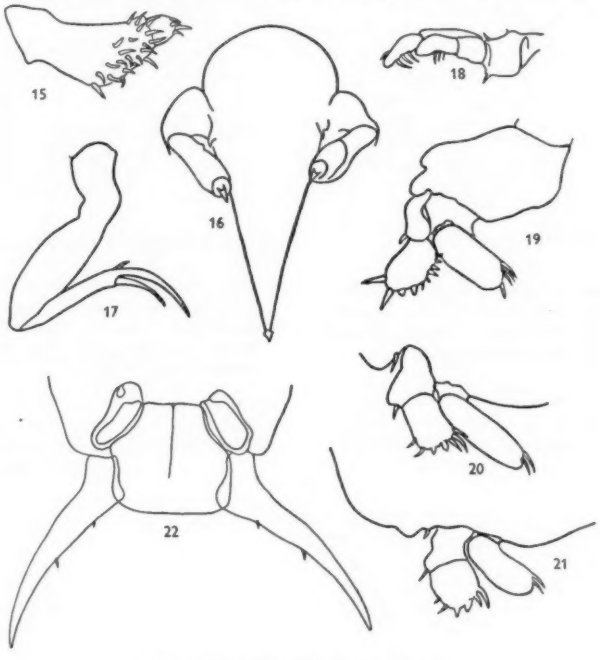


Figs. 7–12. *Dissonus furcatus* n.sp.

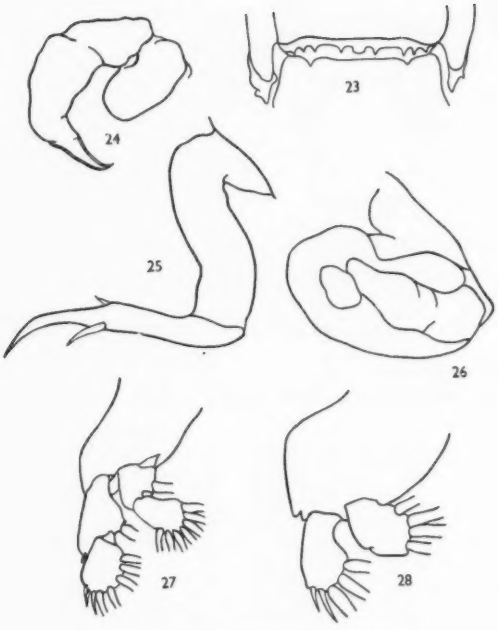
Fig. 7. Second antenna of male. Fig. 8. Mouth cone and first maxillae of male. Fig. 9. Second maxilla of female. Fig. 10. Maxilliped of female. Fig. 11. First swimming leg of female. Fig. 12. Fourth swimming leg of male.



Figs. 13–14. *Pandarus niger* n.sp.
Fig. 13. Female, dorsal view. Fig. 14. Copepodid male, dorsal view.

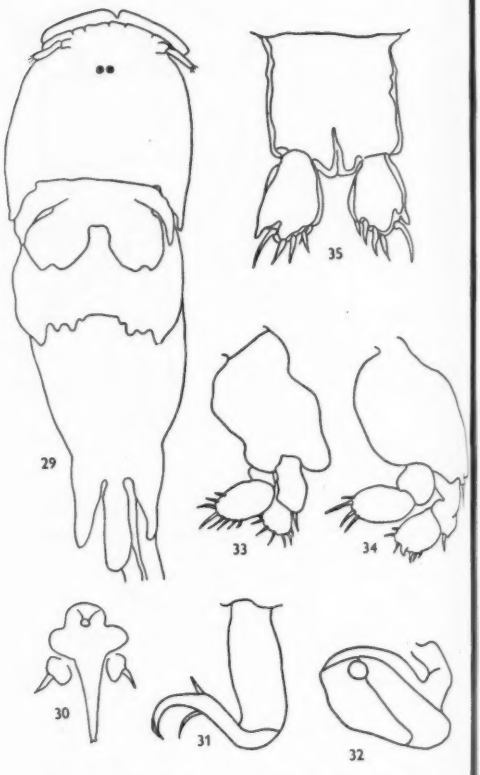


Figs. 15–22. *Pandarus niger* n.sp.
Fig. 15. First antenna of female. Fig. 16. Mouth cone and first maxillae. Fig. 17. Second maxilla.
Figs. 18–21. First to fourth swimming legs. Fig. 22. Abdomen and anal laminae.



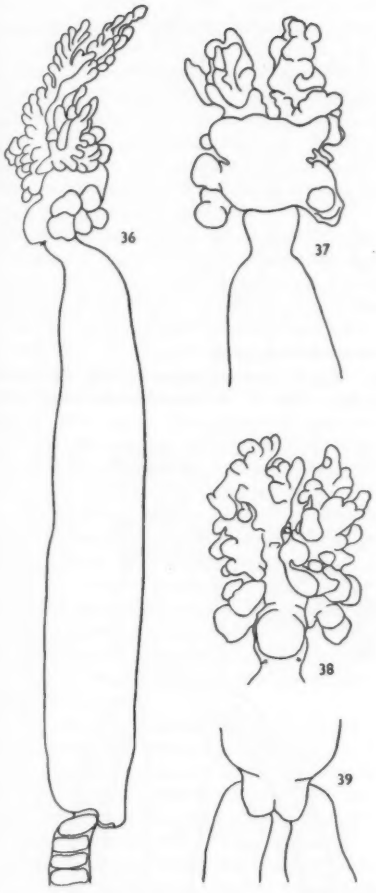
Figs. 23–28. *Pandarus niger* n.sp.

Fig. 23. Posterior margin of cephalothorax of male. Fig. 24. Second antenna and adhesive pad. Fig. 25. Second maxilla. Fig. 26. Maxilliped. Fig. 27. Second swimming leg. Fig. 28. Fourth swimming leg.



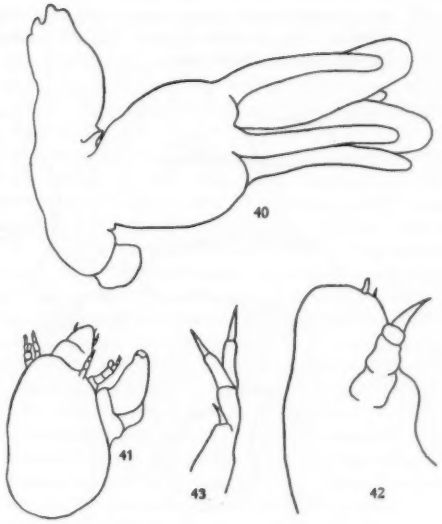
Figs. 29–35. *Pseudopandarus gracilis* n.g., n.sp.

Fig. 29. Female, dorsal view. Fig. 30. Mouth cone and first maxillae. Fig. 31. Second maxilla. Fig. 32. Maxilliped. Fig. 33. Second swimming leg. Fig. 34. Third swimming leg. Fig. 35. Abdomen and anal laminae, ventral view.



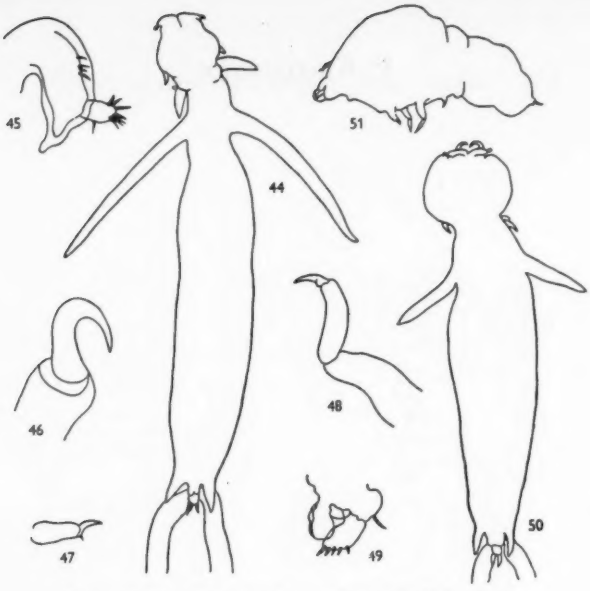
Figs. 36–39. *Cardiodectes medusaeus* Wilson.

Fig. 36. Female, lateral view. Fig. 37. Anterior region, dorsal view. Fig. 38. Anterior region, ventral view. Fig. 39. Posterior region showing the post-abdomen and bases of egg-strings, dorsal view.



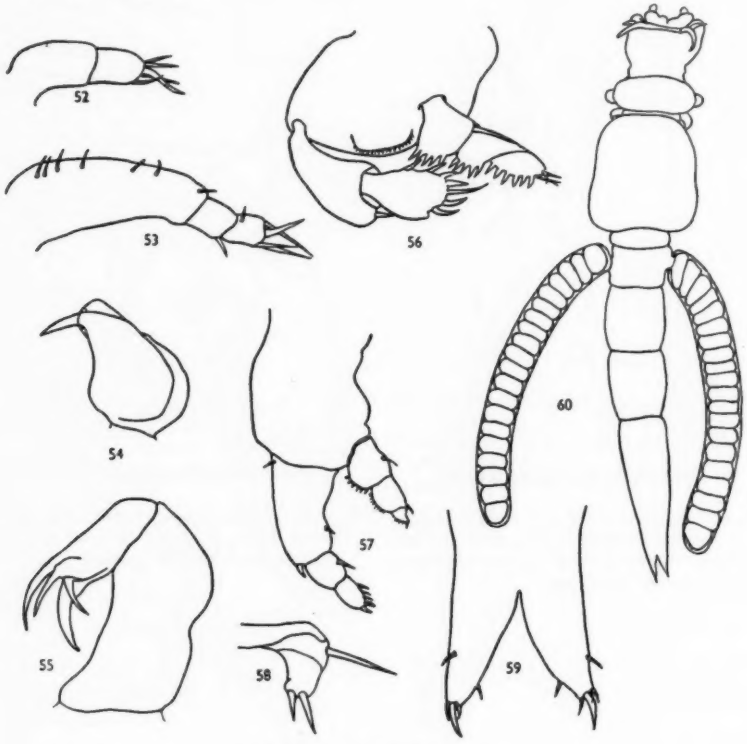
Figs. 40–43. *Clavellopsis appendiculata* n.sp.

Fig. 40. Female, lateral view. Fig. 41. Male, dorso-lateral view. Fig. 42. Second antenna of female. Fig. 43. First maxilla of male.



Figs. 44–51. *Protochondracanthus psettodis* n.g., n.sp.

Fig. 44. Adult female, dorsal view. Fig. 45. First antenna. Fig. 46. Second antenna. Fig. 47. Second maxilla. Fig. 48. Maxilliped. Fig. 49. Second swimming leg. Fig. 50. Juvenile female, dorsal view. Fig. 51. Male, lateral view.



Figs. 52–60. *Lamproglena* sp.

Fig. 52. Second antenna. Fig. 53. First antenna. Fig. 54. Second maxilla. Fig. 55. Maxilliped. Fig. 56. First swimming leg. Fig 57. Fourth swimming leg. Fig. 58. Fifth leg. Fig. 59. End of abdomen. Fig. 60. Female, dorsal view.

spine twice as long as the inner (Fig. 9). Maxillipeds stout, their strongly curved terminal claw shutting against a raised pad on the proximal part of the basal joint (Fig. 10). All four pairs of thoracic limbs biramous, rami of the first pair two-jointed (Fig. 11), of the others three-jointed. Fifth legs, placed at the corners of the genital segment, are in the form of small papillae tipped with four setae, three long and the fourth minute.

Total length (exclusive of egg-strings), 5·5 mm.

Description of the male (Fig. 6). General appearance like that of the female but slender. Abdomen comparatively longer and anal laminae larger. The bifurcated spines on the ventral surface of the genital segment scattered in an arc occupying almost the anterior half of the segment.

Appendages similar to those of the female; but second antennae carrying an additional spine on the inner curve of the hook (Fig. 7). Each leg of the fifth pair represented by two setae placed about midway on the lateral margin of the genital segment, and each leg of the sixth pair by a small seta at the posterior corner of the segment.

Total length, 4·5 mm.

Remarks. The genus *Dissonus* was created by Wilson (1906) for a single species from an unknown host from Ceylon. The present record therefore provides from the same locality a second species in this genus. *D. furcatus* has a longer cephalothorax, abdomen and genital segment than the type species *D. spinifer* Wilson. The strong spines on the ventral surface of the genital segment, which, as Wilson remarked, are admirably suited to preventing the parasite from slipping off the host, are here reduced in number and area of distribution, but this reduction is compensated for by their forked character and arrangement in an arc.

The genus may now be redefined as follows: first thoracic segment fused with the head; second, third and fourth thoracic segments free; only the second segment provided with lateral plates. Ventral surface of genital segment with short, stout, backwardly directed spines. Antennae and mouthparts like those in the Caliginae. First maxilla longer than the mouth cone, with a ventral papilla near its base, and a strong spine close to its tip. Furca absent. The four pairs of swimming legs biramous; rami of the first pair two-jointed, of the remaining pairs three-jointed. Female with fifth legs at the posterior corners of the genital segment, male with fifth legs on the lateral margins and sixth legs at the posterior corners of the genital segment. Abdomen one-jointed in both sexes. Anal laminae with four large plumose setae.

(3) **Pandarus niger** n.sp.

Host and record. Three females and a copepodid male were found on the skin of a carcharinid shark.

Description of the female (Fig. 13). Cephalothorax triangular, narrow in front, but broadening behind to twice its width at the front end; frontal plates wide; posterior margin toothed.

Dorsal plates of the second and third thoracic segments extending to more or less an equal distance posteriorly. Plates of the third thoracic segment, wider than those of the second, separated from each other by a deep median incision. Plates of the fourth thoracic segment fused to form a single rectangular plate overlying more than half the genital segment. Dorsal plates of the genital segment produced posteriorly into slightly bifid lobes on either side of the abdominal plate. Anal laminae large, pointed processes with two or three spines on the inner margin (Fig. 22).

First antennae with setae at the distal ends of both joints (Fig. 15). Second antennae, hooks of moderate size. Adhesive pads as usual in the genus. Mouth tube an elongated cone. First maxilla with a terminal spine. Terminal claw of the second maxilla armed with two spines (Fig. 17). Maxillipeds stout. Thoracic legs biramous, each ramus of two joints (Figs. 18–21).

Dorsal surface of the body brownish black except for a light, nearly rectangular, area in the region of the eyes. Eyes inconspicuous. Anal laminae also uncoloured.

Total length (exclusive of egg-strings), 6·5 mm.

Description of the copepodid male (Fig. 14). Cephalothorax about as wide as long, a little more than one-third the entire length. Frontal plates broad and prominent. Posterior ends of the lateral lobes of the cephalothorax extending backwards more than half-way down the second thoracic segment. Posterior margin of the cephalothorax (Fig. 23) with serrations corresponding in number and arrangement with those of the female. Dorsal plates of the first thoracic segment very small, inconspicuous. Second thoracic segment with pointed processes extending backwards about half-way along the sides of the third segment. Genital segment flask-shaped. Abdomen indistinctly two-jointed. Anal laminae broad, each lamina furnished with four setae.

Appendages similar to those of the female except that the rami of the fourth swimming legs at this stage are apparently one-jointed and that the setae on all the swimming legs are more normal in form and arrangement (Figs. 27, 28).

Colour a golden yellow.

Total length, 5 mm.

Remarks. The strongly developed anal laminae constitute an outstanding feature of the female, recalling most nearly those of *Pandarus satyrus* Dana. In the copepodid male here described spermatophore receptacles are not in evidence, and the conditions of the first joint of the abdomen and the rami of the fourth swimming legs are very similar to those of the

sixth copepodid stage male of *P. cranchii* described by Wilson (1935).

The female of this new species resembles that of *P. bicolor* Leach in the shape of the cephalothorax and in having its two anterior thoracic segments so far fused as to have a common quadripartite dorsal plate. The males of these two species also agree in not possessing conspicuous dorsal plates on the first thoracic segment.

(4) **Pseudopandarus gracilis** n.g., n.sp.

Host and record. Three females were found on the skin of a 'large dogfish'.

Description of the female (Fig. 29). Body elongate, three times as long as broad. Cephalothorax semi-elliptical, a little less than one-third the entire length. Frontal plates distinct. Eyes placed at a point about a third of the length of the cephalothorax from its anterior end. Lateral lobes of the cephalothorax produced posteriorly into triangular projections.

Dorsal plates of the second thoracic segment triangular; those of the third segment broad plates covering about half the dorsal plate of the fourth segment. This latter plate about as wide as the cephalothorax, its posterior margin with small symmetrical lobes. Dorsal plate of the genital segment elongated, narrowing a little posteriorly, its hinder margin produced into three prominent lobes, one median oval and two lateral triangular. Abdomen visible ventrally only, as a single large joint bearing two broad anal laminae. Each lamina with a spine on its outer margin and bearing, in addition, five spine-like setae which decrease in size from the outer to the inner margin (Fig. 35).

First antennae small two-jointed; proximal joint indistinct, second joint longer, bearing terminal setae. Second antennae two-jointed hooks. Mouth cone having a moderately wide base and tapering distally (Fig. 30). First maxillae consisting each of a short base tipped with a strong spine. The claw-like terminal joint of the second maxilla bearing two spines, one short and anterior in position, the other longer and posterior (Fig. 31). Maxillipeds stout and terminating in a strong claw shutting against a knob-like swelling (Fig. 32). All four pairs of swimming legs biramous, rami two-jointed (Figs. 33, 34).

Colour a uniform yellow.

Total length (exclusive of egg-strings), 4 mm.

Generic characters of the female. Cephalothorax, thoracic segments and appendages as in *Pandarus*. Dorsal plate of the genital segment elongated, narrowing a little posteriorly, its hinder edge produced into three lobes, one median and two lateral; the median lobe extending over and beyond the abdomen which is therefore visible ventrally only, as a single joint bearing a pair of broad anal laminae armed with spine-like setae. No separate abdominal plate.

(5) **Cardiodectes medusaeus** (Wilson)

Host and record. Several females were obtained from specimens of *Anchoviella hamiltoni* (Gray) hawked for sale in Colombo. In this particular catch of fish the percentage of infestation appears to have been very heavy. Almost every fish harboured one or more of these parasites. In a lot of fifteen infected fish, six had one parasite each, five had two parasites each and the other four carried three parasites each. Most of the parasites were attached to the host at the middle of the base of the caudal fin on one side, sometimes on both sides. The base of the anal fin was an additional site of attachment.

Remarks. The distribution and the distinctive features of the four known species of *Cardiodectes* have been summarized by Schuurmans Stekhoven (1937). He was of opinion that the occurrence of the genus in the Indian Ocean needed confirmation, as he felt that the only record (that of a *C. medusaeus* taken near Mauritius) from this region was inadequate. This confirmation is here provided. *Anchoviella hamiltoni* is a new host for this species.

Dimensions of the female (in mm.): cephalothorax and neck, 1·5; trunk, 4·5; egg-strings, up to 18; entire length (exclusive of egg-strings), 6 (Figs. 36–39).

(6) **Brachiella gulosa** Wilson

Host and record. Three females, two of them with attached males, were obtained from the mouth of *Trichiurus savalla*.

Remarks. This makes the only record of the species since it was originally described by Wilson (1915), the host fish in that instance being *Sciaenops ocellata*. The present specimens have the dorsal pair of posterior processes of the genital segment in the female longer than the ventral pair; in the original specimens the ventral processes are the longer.

(7) **Clavellopsis appendiculata** n.sp.

Host and record. Five females with a male attached to one of them were obtained from the gill arches of the Indian dorab, *Chirocentrus dorab*.

Description of the female (Fig. 40). Cephalothorax slightly longer than the trunk, stout in fully developed, but moderate in younger specimens. Head somewhat flattened dorso-ventrally, anterior edge squarish. Trunk pear-shaped, also somewhat flattened dorso-ventrally and produced into two pairs of backwardly directed cylindrical processes, the dorsal processes arising laterally while the ventral processes arise close together near the middle line. Abdominal process minute, more distinct in younger specimens.

First antenna directed ventrally, four-jointed, with two terminal setae. Second antennae have each an

indistinctly jointed endopod provided with two or three setae at the tip and a ventrally directed exopod consisting of three joints, the short terminal joint tipped with a spine (Fig. 42). First maxilla bipartite, its palp with a single spine. Second maxillae completely fused with no trace of the line of fusion. Maxillipeds stout, having a short projection at a third of the distance from the base.

Dimensions (in mm.): cephalothorax, 2; trunk, 1·75; posterior processes, 1·7; entire length, 5·45.

Description of the male (Fig. 41). Shape oval. First antenna four-jointed with terminal setae of which one is spine-like. Second antenna biramous, sympod of three joints, endopod of one oval joint and exopod of two joints with terminal tuft of setae. Mouth cone large, projecting its entire length in front of the thorax and turned ventralwards at an angle to the long axis of the body. First maxillae similar to those of the female, their tips reaching to the anterior end of mouth cone (Fig. 43). Second maxillae and maxillipeds stout claws. No anal laminae.

Total length, 0·46 mm.

Remarks. The structure of the bulla is not distinct. The long posterior processes of the female are remarkable for this genus. Thompson & Scott (1903) have recorded *Brachiella thynni* from the gills of the Indian dorab. The correctness of their identification has already been questioned (Kirtisinghe, 1935), and it now appears that they mistook the species here described for *B. thynni* on account of the long posterior processes common to both species.

(8) **Protochondracanthus psettodis** n.g., n.sp.

Host and record. Several females with males attached to some of them were obtained from the gill filaments of the flatfish, *Psettodes erumei*.

Description of the female (Fig. 44). Body elongate. Head oval, longer than wide, a short median suture at its anterior end dorsally, and a pair of postero-lateral cephalic barbs.

Thoracic segments fused, not marked off from the head except for a slight constriction. Thoracic region a little narrower than the head; about two-thirds of the way from its anterior end it bears a pair of prominent, postero-laterally directed processes with tapering ends. Thorax passes imperceptibly into the broader, cylindrical trunk region. Genital segment prolonged at its posterior corners into two short conical processes. Abdomen small, one-jointed, carrying a pair of conical caudal rami each about as long as the abdominal joint.

First antenna two-jointed, basal joint curving backwards and tapering to a blunt point, anterior and lateral to which the short, setiferous second joint is inserted (Fig. 45). Second antennae strong curved claws (Fig. 46). First maxilla finely serrated on the outer margin of the terminal joint. Second maxilla two-jointed with a small spine near the base of the terminal joint (Fig. 47). Maxillipeds three-jointed (Fig. 48). First thoracic limb bipartite, one ramus directed forwards and much smaller than the other, postero-laterally directed ramus. Second thoracic legs (Fig. 49) placed immediately behind the bases of the first legs, very small, biramous; each ramus one-jointed, the endopod an oval lobe, while the exopod bears five short, spine-like setae along its postero-lateral border.

Ovisacs may be longer than the entire body.

Total length (exclusive of ovisacs), 7 mm.

Description of the male (Fig. 51). Body pyriform. One or two thoracic segments partially free. Abdomen one-jointed, with a pair of conical caudal rami.

First antennae each three-jointed bearing terminal setae; slightly dorsal and posterior in position to the claw-like second antennae which are anteriorly situated. Second maxillae and maxillipeds similar to those of the female. A minute pair of thoracic limbs present.

Total length, 0·3 mm.

Remarks. The females include individuals (Fig. 50) which have not attained the full-grown condition. They are, however, not immature as is evidenced by their possession of ovisacs and attached males.

The host *Psettodes* is the most primitive genus of the Heterosomata (flatfishes). The fact that its parasitic copepod shows features intermediate between those of *Chondracanthus*, not found on flatfishes, and those of *Acanthochondria*, the typical gill parasite on other flatfishes, is not without interest.

Genus diagnosis. Female with oval head separated from the thorax by a slight constriction only; all the segments fused into an elongated body having only a trace of segmentation in the anterior thoracic region; thoracic region narrower than the head and trunk; cephalic barbs and lateral processes on the body but no dorsal or ventral processes; abdomen one-segmented, carrying a pair of conical caudal rami; second antennae prehensile; terminal joint of first maxilla spine-like faintly toothed on its outer margin; two pairs of biramous swimming legs; second pair very small, its exopods with spine-like setae.

Male with a head larger than the rest of the body; all the segments almost completely fused; faint traces of segmentation in the thoracic region; abdomen a minute papilla bearing a pair of conical caudal rami; antennae and mouthparts as in female; one pair of leg rudiments (first swimming legs) discernible.

(9) **Lamproglena** sp.

Host and record. Eight females were obtained from the gill filaments of the snakehead, *Ophiocephalus striatus*.

Description of the female (Fig. 60). Head separated by deep lateral sinuses into anterior and posterior regions; posterior region shorter but slightly wider than the anterior region, giving the appearance of a separate segment in a dorsal view owing to the dorsal bulging of this region. First thoracic segment free, very short and overlapped by the head and by the second thoracic segment; in width equal to the narrowest part of the head. Second to fourth thoracic segments fused to form a region nearly rectangular in shape having a greater width than that of the head. Fifth segment free, short, as wide as the first thoracic segment. Genital segment of the same width as the fifth segment but longer. Abdomen of three segments, first two segments equal in size and each as long as the fifth and genital segments combined; third segment longer, making up half the total length of the abdomen.

First antenna three-jointed, basal joint long, a few setae on its anterior border; second and third joints short, a few setae at the tip of the terminal joint (Fig. 53). Second antenna short, two-jointed, with a few terminal setae (Fig. 52). Second maxillae powerful claws (Fig. 54). Maxilliped two-jointed, terminal joint with four claws (Fig. 55). First four pairs of thoracic legs biramous, exopodites of the first pair two-jointed, endopodites three-jointed; rami of the next three pairs three-jointed (Figs. 56 and 57). Fifth leg uniramous, with a spine-like seta on the base and two terminal setae (Fig. 58).

Egg-strings reaching to the tip of the abdomen.

Total length, 3·4 mm.

Remarks. This form is not represented among the species described from the Soviet Union by Markewitsch (1936). However, Yamaguti (1936) and Yu (1937) have described two other species, *Lamproglena ophiocephali* and *L. chinensis*, on the same genus of host fish, *Ophiocephalus*, from Japan and China respectively. As these two publications have not been accessible the identification of the present specimens has had to be left open. This record shows the most southerly distribution yet of a *Lamproglena*.

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A METHOD FOR ESTIMATING THE TOXIC EFFECT OF CONTACT INSECTICIDES ON MOSQUITOES AND HOUSE-FLIES

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INTRODUCTION

It is evident that the efficiency of D.D.T. residual spraying of buildings as a control measure against mosquitoes and flies depends on the frequency with which the insect vector comes in contact with the sprayed surface, the duration of contact, and the subsequent length of life of the vector. This last can be established experimentally, and can probably be used to measure the efficiency of various contact insecticides against a given species, or alternatively, the susceptibility of different species to the toxic effect of a given insecticide.

The experiments reported below were carried out with the purpose of establishing the nature of the correlation that may exist between duration of contact with an insecticide-sprayed surface, and subsequent survival of Anophelines and house-flies.

Individual Anophelines and flies were induced to come in contact for a limited time with a glass plate sprayed with D.D.T. solution. The survival of exposed individuals in standard conditions of humidity and temperature was recorded. Group-average length of life was calculated and compared with that of the controls. Throughout the experiments chemically pure D.D.T. in commercial kerosene was used.

METHODS AND APPARATUS

There are two basic problems to surmount:

(1) To devise an apparatus whereby a known amount of insecticide can be distributed evenly over a known surface.

(2) To devise an apparatus which will ensure that the experimental insect is brought into contact with the sprayed surface in such a way that the duration of contact can be measured. This apparatus must be designed separately for each type of insect, owing to differences in habits. For instance, it is difficult to induce a fly to alight on a surface for a specified length of time, whereas it is possible to induce it to walk over the surface for the required length of time.

The spraying apparatus consists of a metal drum slowly rotating around the vertical axis. To this drum glass plates 13·5 × 13·5 cm. are fastened. A cone of fine spray is directed at the apparatus from a sprayhead which is attached to a pressure reservoir, 125 cm. away from the rotating drum. As the plates pass through the cone of spray they are completely and evenly covered. The full revolution of the drum is completed in 5 sec., and the spraying pressure is 40 lb./sq.cm. Under these conditions every glass plate is uniformly covered by 2 mg. of solution per sq.cm. The sprayed glass plates are subsequently kept for 24 hr. at 37° C. and 40 % r.h., to ensure quick and uniform evaporation of the solvent. The plates, after drying, were used for experiments during the same day.

In order to make mosquitoes come in contact with the sprayed surface, an apparatus consisting of a cylindrical glass chamber open at one end and rotating about its long axis was used. The sprayed glass plates were placed over the open end, and represented the only non-rotating surface on which the mosquitoes, introduced into the chamber, could alight, when the cylinder was made to revolve.

In order to make house-flies come in contact with the sprayed surface, a glass tunnel, of triangular cross-section and closed at both ends, was constructed from a glass base-plate, and two glass strips, in the manner of a 'cloche'. The tunnel was 11 cm. long, base 1 cm. in breadth and 1 cm. from apex to base. The glass strips were sprayed in the same way as the glass plates. The tunnel is placed on a wooden board, balanced in the middle. Flies are introduced into the tunnel, by temporarily removing one of the strips which seal the ends. By changing the angle of inclination of the board, and the illumination, the flies are induced to walk backwards and forwards continuously in the tunnel.

Ten Anophelines or house-flies were tested on each glass plate or tunnel. After the test, the insects were kept in cages uncontaminated with insecticide, at 24–27° C. and 75–85 % r.h. The number of Anophelines knocked down and unable to rise when the cage was shaken was recorded every 3 hr. during the first 12 hr. after the test. Further observations were carried out every 12 hr. The number of flies knocked down was recorded every half hour during the first 3 hr., and every 3 hr. thereafter. The insects were given no food or water during the observation period. For control tests, the insects were induced to come in contact with a surface sprayed with kerosene only, for the same

length of time, and were observed under the same conditions.

In the succeeding paragraphs it is convenient to use the following terms in the following ways:

(1) 'Knocked down' insects are described as dead. Actually, in no case was recovery of a knocked-down Anopheline or house-fly observed.

(2) Duration of life is the period between the end of contact with the insecticide, and the time of knockdown.

(3) Surviving insects are those not yet knocked down at any time of observation.

Anopheles *tests. Series* 1 (*Tables* 1 *and* 2)

Object: To discover the duration of life after different lengths of time in contact with the insecticide.

Material: Unfed, non-gestating females of *Anopheles sacharovi*.

Solution: 5% D.D.T. in commercial kerosene.

Strength: 2 mg. solution per sq.cm. of surface.

Contact time: 2·5, 5 and 10 min.

Control: Contact for 10 min. with surface sprayed with the same quantity of kerosene only.

Table 1

Survival of *Anopheles sacharovi* (meagre females) after contact with

	D.D.T.-sprayed surface			Kerosene-sprayed surface
Duration of contact ...	2·5 min. (5 tests)	5 min. (10 tests)	10 min. (4 tests)	10 min. (5 tests)
Hr. after contact	No. of Anophelines alive			
0	50	100	40	50
3	38	47	18	50
6	34	34	6	48
9	30	20	0	48
12	29	9	—	43
24	17	0	—	33
36	7	—	—	21
48	5	—	—	13
60	3	—	—	11
72	3	—	—	2
84	2	—	—	0
96	1	—	—	—
108	0	—	—	—
Calculated average life of Anophelines	21·09 hr.	5·205 hr.	3·3 hr.	35·91 hr.

Table 2. *Number of Anophelines that died between two successive times of observation. Percentage of the number alive at the beginning of the period*

	D.D.T.-sprayed surface						Kerosene-sprayed surface	
Duration of contact ...	2·5 min.		5 min.		10 min.		10 min.	
Observation (hr.)	No.	%	No.	%	No.	%	No.	%
0	12	24	53	53	22	55	0	0
3	4	11	13	28	12	63	2	4
6	4	12	14	41	6	100	0	0
9	1	3	11	50	—	—	5	10
12	12	41	9	100	—	—	10	23
24	10	55	—	—	—	—	12	37
36	2	29	—	—	—	—	8	39
48	2	40	—	—	—	—	2	15
60	0	0	—	—	—	—	9	82
72	1	33	—	—	—	—	2	100
84	1	50	—	—	—	—	—	—
96	1	100	—	—	—	—	—	—
108								

Statistical analysis of data by PROF. R. BACHI, *Hadassah Hospital, Jerusalem*

Comparison and analysis of results obtained with each test-group of ten Anophelines (from Table 1)

Duration of contact ...	2·5 min.			5 min.			10 min.		
Group	Calculated average life (hr.)	S.E. $\sigma/\sqrt{n}$	S.D.	Calculated average life (hr.)	S.E. $\sigma/\sqrt{n}$	S.D.	Calculated average life (hr.)	S.E. $\sigma/\sqrt{n}$	S.D.
1	18	4·8	10·74	3·9	0·15	0·49	3·3	0·22	0·45
2	18·3	—	—	5·05	—	—	3·6	—	—
3	43·2	—	—	5·05	—	—	3·3	—	—
4	13·05	—	—	4·75	—	—	3·0	—	—
5	19·5	—	—	5·05	—	—	—	—	—
6	—	—	—	5·35	—	—	—	—	—
7	—	—	—	5·65	—	—	—	—	—
8	—	—	—	5·35	—	—	—	—	—
9	—	—	—	4·75	—	—	—	—	—
10	—	—	—	5·35	—	—	—	—	—

Standard deviation $\sigma = \sqrt{\Sigma (y)^2/n}$.
y, difference between average life of a group and average life of Anophelines in all groups.
n, number of tests.

Analysis of totals as summarised in Table 1

2·5 min. contact (5 tests)			5 min. contact (10 tests)			10 min. contact (4 tests)		
S.D.	S.E.	C. of V.	S.D.	S.E.	C. of V.	S.D.	S.E.	C. of V.
22·35	10·02	1·06	5·039	1·6	0·96	3·24	1·62	0·98

Standard deviation $\sigma = \sqrt{\Sigma (x)^2 f/\Sigma f}$.
x, length of life of each Anopheline—average life of all Anophelines.
f, number of Anophelines dead between two successive times of observation.
n, number of tests.
Standard error $= \sigma/\sqrt{n}$.
Coefficient of variation: σ/M.
M, average life of all Anophelines.

Series 2. Meagre *Anopheles sacharovi* females tested on glass plates sprayed with D.D.T. kerosene solution of different strengths, containing respectively 0·25, 1, 2·5 and 5% of D.D.T.
Quantity: 2 mg. of solution per 1 sq.cm.
Contact time: 5 min.

Table 3

Hr. after contact ...	0	3	6	9	12	24	36	48	
D.D.T. Solution %	Number of Anophelines alive								Calculated average life of Anophelines (hr.)
0·25%	10	8	6	5	4	3	2	0	16·2
0·5%	10	7	5	4	2	0	—	—	7·8
1%	10	5	3	2	1	0	—	—	5·25
2·5%	10	4	2	2	1	0	—	—	4·65
5%	10	4	3	2	1	0	—	—	4·95

Series 3. *Anopheles sacharovi* females with blood and eggs were tested on plates with 5% D.D.T. solution in kerosene.
Quantity: 2 mg./1 sq.cm.
Contact time: 5 min.

Table 4. *Survival of* Anopheles sacharovi *females in different physiological state after contact with D.D.T.-sprayed surface*

Hours after contact	No. of Anophelines alive: With eggs and blood	Meagre
0	10	10
3	7	5
6	6	3
9	5	2
12	3	1
24	1	0
36	1	—
48	0	—
Calculated average life (hr.)	11·55	5·25

In these experimental conditions the average life after contact is within large limits independent of the D.D.T. content (1–5 %) of the solution used for spraying, if the contact time is more than 5 min. *A. sacharovi* females with blood and eggs seem to be less susceptible to the toxic action of D.D.T.

House-fly tests

Flies used in these experiments were *Musca domestica* var. *vicina* reared in the laboratory. Meagre females and males were 24 hr. old and females with eggs 1–2 weeks old. The old flies were fed on glucose in water and yeasts. The meagre ones received only glucose in water 12 hr. before the test. No food or water was supplied after the test.

Table 5. *The survival of* Musca domestica *var.* vicina *after* 90 *sec. contact with surfaces sprayed with* 5 % D.D.T. *solution in kerosene and kerosene only.*

Time after contact	24 hr. old females: D.D.T. (5 tests)	24 hr. old females: Kerosene (1 test)	24 hr. old males: D.D.T. (4 tests)	24 hr. old males: Kerosene (1 test)	Females with eggs: D.D.T. (5 tests)	Females with eggs: Kerosene (1 test)
	No. of flies alive					
0 min.	50	10	40	10	50	10
30 min.	32	10	21	10	35	10
60 min.	14	10	10	10	23	10
90 min.	3	10	3	10	13	10
120 min.	0	10	0	10	8	10
150 min.	—	10	—	10	5	10
180 min.	—	10	—	10	2	10
210 min.	—	10	—	10	0	10
6 hr.	—	8	—	7	—	9
9 hr.	—	7	—	6	—	8
12 hr.	—	5	—	3	—	7
24 hr.	—	0	—	0	—	0
Calculated average life (hr.)	0·7	12·75	0·67	10·65	1·1	14·85

Individual differences in length of life were observed in Anophelines on the same group. The death-rate and average life in different random groups of ten *A. sacharovi* meagre females, after contact with identically treated D.D.T.-sprayed surfaces, depend on the duration of contact and are sufficiently constant to be comparable, provided the duration of contact is not less than 5 min. With 2·5 min. contact the results obtained with various groups differed considerably. In this case the toxic effect was very slight. The death-rate in all groups of Anophelines treated was higher during first 3 hr. after the contact. With contact of 2·5 min. only, after the first 12 hr. the death-rate of treated Anophelines is similar to that of controls.

The flies were induced to walk inside the glass prism for 90 sec., their legs only coming into contact with D.D.T.-sprayed surface. After repeated trials, 90 sec. was found to be the most convenient duration of exposure in fly tests. 'The time that it takes to let out the fly from the apparatus varies from one fly to another. It may be 5 or as much as 15 sec. The difference is important if the exposure is of shorter duration. With longer exposures, the flies die so quickly that first flies may be knocked down before the test of the whole group is completed. Two separate observers would be necessary in this case.'

Results of these tests are summarized in Tables 5 and 6.

Statistical analysis of data by PROF. R. BACHI, *Hadassah Hospital, Jerusalem*

Comparison and analysis of results obtained with each test-group of ten flies (Table 5).

	24 hr. old females			24 hr. old males			Females with eggs		
Group	Calculated average (hr.)	S.E.	S.D.	Calculated average (hr.)	S.E.	S.D.	Calculated average (hr.)	S.E.	S.D.
1	0·85	0·03	0·077	0·6	0·03	0·056	1·15	0·06	0·14
2	0·65	—	—	0·65	—	—	1·0	—	—
3	0·75	—	—	0·75	—	—	1·2	—	—
4	0·70	—	—	0·70	—	—	1·2	—	—
5	0·75	—	—	—	—	—	1·0	—	—

Standard deviation $\sigma = \sqrt{\Sigma (y)^2/n}$.
y, difference between average life of a group and average life of flies in all groups.
n, number of tests.

Analysis of totals summarized in Table 5.

24 hr. old females			24 hr. old males			Females with eggs		
S.D.	S.E.	C. of V.	S.D.	S.E.	C. of V.	S.D.	S.E.	C. of V.
0·5	0·2	0·7	0·47	0·23	0·7	0·85	0·38	0·77

Standard deviation $\sigma = \sqrt{\Sigma (x)^2 f/\Sigma f}$.
x, length of life of each fly—average life of all flies.
f, number of flies dead between two successive times of observation.
n, number of tests.
Standard error $= \sigma/\sqrt{n}$.
Coefficient of variation, σ/M.
M, average life of all flies.

Table 6. *The survival of* 24 *hr. old females of* Musca domestica *var.* vicina *after* 90 *sec. contact with surfaces sprayed with* 2 *mg./sq.cm. D.D.T. solutions of various strengths*

D.D.T. solutions (%) ...	5		2·5		1		0·5		0·25
Test no.	I	II	I	II	I	II	I	II	I
Time after contact	No. of flies alive								
0 min.	10	10	10	10	10	10	10	10	10
30 min.	6	6	6	7	7	8	9	10	10
60 min.	2	3	3	3	4	4	7	8	8
90 min.	0	1	1	2	3	3	5	6	6
120 min.	—	0	0	0	2	2	5	6	4
150 min.	—	—	—	—	1	2	4	5	4
180 min.	—	—	—	—	0	1	3	4	3
4 hr.	—	—	—	—	—	0	2	3	2
5 hr.	—	—	—	—	—	—	2	2	2
6 hr.	—	—	—	—	—	—	1	1	1
7 hr.	—	—	—	—	—	—	1	0	0
8 hr.	—	—	—	—	—	—	0	—	—
Calculated average life (hr.)	0·65	0·75	0·75	0·85	1·1	1·2	2·5	2·9	2·5

The house-flies, like the Anophelines, show individual differences in resistance to D.D.T. In various test groups of ten flies the death-rate and survival after the same duration of contact are sufficiently constant to be comparable, and within certain limits are independent of the D.D.T. content of solution used (2·5–5 %). No differences in behaviour were observed between groups of males and females of the same age; older flies with eggs seems to be more resistant to the action of D.D.T.

SUMMARY

A method for comparing the lethality effect of contact insecticides on mosquitoes and house-flies is proposed.

It is based on the comparison of the death-rate and the length of survival of insects after a contact for a very short and exactly measured time with a surface sprayed with a known concentration of the insecticide.

It is suggested that the same method may be used to compare the susceptibility of various species of mosquitoes and flies to the toxic action of a contact insecticide.

Some of the methods and apparatus were worked out and constructed by the Staff of No. 1 Malaria Field Laboratory under the command of the senior author. The following Officers and N.C.O.'s of the Royal Army Medical Corps were especially active in this work, and acknowledgement is made of their valuable contributions: Major T. T. Macan, Major Fox, Lt. MacDonald, Staff-Sgt. (later Major) Iles, Cpl. Mortimer.

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ON SOME ABNORMALITIES IN *HYALOMMA SAVIGNYI**

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(With 7 Figures in the Text)

Many workers have described abnormal forms and structures in ticks. Neuman (1899) was the first who recorded some abnormal Ixodidae. Brumpt (1934) reviewed the literature and recorded some new interesting abnormalities. The posterior region of the body of one of the specimens described by Brumpt had two ani and four stigmata. Brumpt considered that only the Ixodidae display abnormal forms, but since then Robinson (1943–4) described abnormalities in laboratory-bred *Ornithodorus moubata*. He also noticed a 'partially twinned' tick which mated, and laid eggs which developed into normal ticks.

In our collection and breedings of ticks some hitherto undescribed interesting abnormalities were noticed.

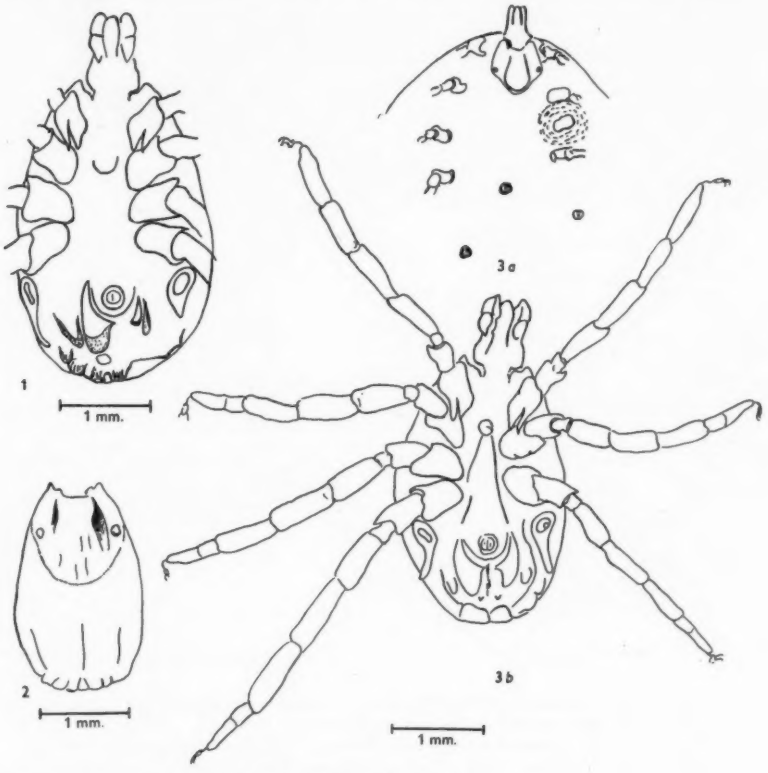

Fig. 1. A laboratory-bred gynandromorph of *Hyalomma savignyi*.
Fig. 2. A pseudogynandromorph of *Hyalomma savignyi*.
Fig. 3. (*a*) Exuvia of nymph of *Hyalomma savignyi*. The third right leg has only a coxa. Note the large scar around the coxa. (*b*) The male tick which emerged from the nymph, *a*.

* This work has been made possible by the Helen Marr Kirby Fellowship granted by the International Association of University Women.

(1) A laboratory-bred male of *Hyalomma savignyi* showing gynandromorphism. One stigma is masculine, the other almost entirely feminine. The anal and adanal shields are greatly reduced on one side. The subanal shield is wanting. The anterior part of the tick is normally masculine (Fig. 1).

(3) It is not rare to find asymmetrical specimens in nature, among them adults with three legs on one side and without a trace of the fourth. One laboratory-bred nymph metamorphosed to an asymmetrical male with only three legs on one side. It is interesting to note that this specimen was injured as a larva, and

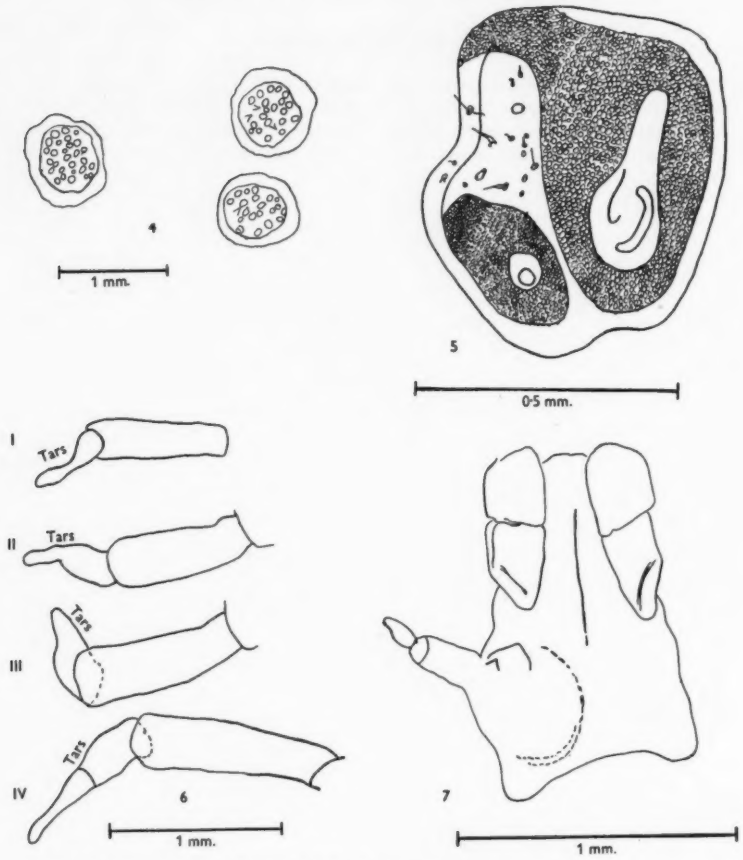

Fig. 4. Areae porosae dorsalis of *Hyalomma savignyi*, doubled on one side.
Fig. 5. A doubled stigma of a female of *Hyalomma savignyi*.
Fig. 6. Four abnormal tarsi of female A57-abn. 1. of *Hyalomma savignyi*.
Fig. 7. A leg-like outgrowing from the basis capituli of *Hyalomma savignyi*.

(2) A pseudogynandromorph was found in Tel-Joseph on 20 October 1945 on cattle. The specimen in question is a male tick, but the anterior part of its scutum exhibits feminine characters. Posteriorly the scutum is typically male (Fig. 2). A specimen exhibiting similar characters was found by Schulze (1923) and described as a gynotropic male. The microscopic examination of our specimen has shown that the genital opening is masculine.

the nymph appeared with a third coxa but without other parts of the third leg. In addition, there was a large scar on the tegument around the basis of the coxa. The absence of the third leg of the nymph and the marked asymmetry (also of internal organs and particularly of those which distribute nutritional material) resulted in an imago whose one half (including the four legs) is larger than the other one (Fig. 3).

(4) In our breedings several specimens with two areae porosae dorsalis on one side were found. The area porosa on the other side of these specimens was normal (Fig. 4).

(5) A case of doubling of the stigma of a female *H. savignyi* was found (Fig. 5).

(6) Several wild females and some of our own laboratory specimens showed abnormal chitinization in their alloscutum. The integument of the alloscutum instead of being thin, clear and uniform showed patches of red brown chitin similar to that of the scutum.

(7) Among the offspring of one female, A 57, found on cattle in Tel-Joseph on 20 October 1945 many ticks had abnormal legs. The tarsus is reduced to a small and degenerated appendix. The pulvillus and claws are absent. Occasionally one or more of the tarsi of these abnormal ticks were normal. Out of 223 ticks bred from the female A 57, thirty-two had at least one abnormal leg (Fig. 6).

(8) One tick of the progeny of female A 57 which had abnormal legs as described above, had on its basis capituli an outgrowth resembling an extremity of a leg. The distal part of the first division of this outgrowth was of a bright colour similar to the articulation of legs of ticks (Fig. 7).

As this abnormality appears in the progeny of female A 57, the offspring of which showed a large number of abnormally developed legs, it might be assumed that the deformation of the legs of the progeny of female A 57, described in paragraph (7), is hereditary.

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A REVIEW OF THE SPECIES OF *DINOBOTHRIUM* (CESTODA), WITH A DESCRIPTION OF A NEW SPECIES

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(With 9 Figures in the Text)

During some investigations on the Basking Shark carried out at Soay, in the Hebrides, in May 1947 by Dr L. Harrison Matthews and Mr H. W. Parker, attention was paid, among other things, to parasites, and a number of examples of the genus *Dinobothrium* were obtained. These were handed over to the writer for study, and proved to include two species, *D. planum* Linton and a much smaller form which appeared to be undescribed. A few months earlier Miss N. G. Sproston had presented to the Museum some examples of *D. planum* collected from a Basking Shark at Plymouth. She also mentioned at the time that she had prepared a paper on the genus (Sproston, 1948) describing two new species, and has since kindly presented the type slides of these to the Museum.

Other material available for study includes specimens of *D. planum* from a Basking Shark, obtained in Japan some 25 years ago by Mr A. V. Insole, and a number of the preparations of *D. septaria* v. Beneden upon which Dr W. N. F. Woodland based his study of this species (Woodland, 1927). These last were presented to the Museum, with much other material, by the Director of the Wellcome Laboratories of Tropical Medicine.

It is not intended in this paper to give full redescriptions of all the known forms, but a study of the relevant literature shows that there is still some difficulty in separating the species, and that a critical review, mentioning only their most distinctive characters, may help to clear up the confusion that has existed within the genus.

Dinobothrium septaria v. Beneden, 1889

Synonyms:

(?) *Diplobothrium simile* v. Beneden, 1889.
D. simile of Lönnberg, 1892, 1899.
Dinobothrium septoria [*sic*] of Lönnberg, 1892.
D. septaria of Lönnberg, 1899.
(?) *D. septaria* of Scott, 1909.
Phyllobothrium septaria of Southwell, 1925 (in part).
Dinobothrium septaria of Woodland, 1927.
D. septaria of Perrenoud, 1931.
D. septaria of Joyeux & Baer, 1936.
(?) *D. keilini* Sproston, 1948 (see below).
[Not *D. septaria* of Mola, 1906; of Masi, 1913; or of Nybelin, 1914.]

So far as adult forms are concerned, the writer agrees, in the main, with the synonymy given for this species by Sproston.* The specific identity of the larval forms described by Linton (1897) and by Dollfus (in Joyeux & Baer, 1936) is uncertain, and will be discussed later. Joyeux & Baer (1936) give *Diplobothrium simile* v. Beneden as a synonym. There can be little doubt that the *D. simile* of Lönnberg (1892, 1899), who described the species from the same host, is identical with *Dinobothrium septaria*. In all probability v. Beneden's original material of *Diplobothrium simile* was of the same species, but it was immature, and the question cannot, therefore, be decided by reference to the anatomy of the segments. Van Beneden, Lönnberg and Scott (1909) all seem to have observed scolices in an extremely contracted as well as in the relaxed condition, and to have been deceived into thinking that they belonged to different species. It is rather strange that Lönnberg should have given, as he did, almost identical descriptions and figures of the anatomy of the two worms, and yet believed them to belong, not only to different species, but to different genera because of the apparent differences in the scolex.

The *Dinobothrium septaria* of Scott (1909) resembles *D. planum* in the size of its scolex, but the host was *Lamna cornubica*, the usual host of *Dinobothrium septaria*. The greater part of Scott's description is quoted from v. Beneden's account of *D. septaria*, but the figure of the scolex shows bothria of the form said by Linton (1922) to be characteristic of *D. plicitum*. This peculiarity, however, has been observed by the writer in some specimens assigned to *D. planum* (see below), and according to Woodland (1927) it also occurs in *D. septaria*.

Van Beneden's original description of *D. septaria* was confined to external characters, and his material appears to have been immature. Lönnberg (1892, 1899) gave some account of the anatomy of mature segments (partly under the name of *Diplobothrium simile*), and detailed descriptions have been given by Woodland (1927) and Perrenoud (1931). From these accounts, and from the writer's examination of Woodland's material, the principal diagnostic features of the species appear to be: (1) the course of the vagina,

* Regarding *D. plicitum* Linton, see below.

which runs forward almost to the anterior end of the segment, and then returns in the middle line to the ovarian complex at the posterior end; (2) the presence of two sphincters on the vagina near its opening (described by Lönnberg and by Perrenoud, and present in Woodland's material, though not mentioned by him); (3) the large size and great relative width of the cirrus-sac, which may measure as much as 1·8 mm. in length and 0·6 mm. in width;* (4) the arrangement of the vitellaria in two lateral ⊃-shaped sheets, external to the excretory canals and extending for some distance towards the middle line dorsally and ventrally.

As regards the characters of the scolex, none of the above-mentioned describers† refers to the presence of conical muscular processes (the 'prongs' of Sproston) on the 'auricles'. A series of sagittal sections of the scolex prepared by Dr Woodland shows, however, that these structures, though very small and not at all prominent, are present to the number usual in the genus, namely, two corresponding to each bothrium, and situated at the outer and median ends of the 'auricle'. Lönnberg (1892) appears to be the only author who has mentioned and figured the minute spines on the convex surfaces of the bothria in this species. Dr Woodland's sections also show these spines, which are extremely fine and closely set.

Host: Porbeagle (*Lamna cornubica*). If *Dinobothrium keilini* Sproston is the same species, the Blue Shark (*Carcharinus glaucus*) is another host.

Dinobothrium keilini Sproston, 1948

The anatomical features of this species strongly suggest that it is identical with *D. septaria*. The vagina follows the course that has been mentioned as characteristic of that species, and has the same two sphincters. Similar very fine and closely set spines are present on the bothria. The arrangement of the vitellaria is not very clearly described by Miss Sproston, but on re-examination of her material it has been found to be the same as in *D. septaria*. Miss Sproston says that 'though *D. keilini* is in many ways very close to the genotype, the character of the scolex is quite distinctive, and taken together with the characters of the vagina and cirrus and the larger testes, vitellaria and eggs, there can be no doubt that it constitutes a new and valid species'. While it is true that the writer has not been able to see any muscular 'prongs' on the auricles in the mounted material of *D. keilini* available, it has already been remarked that these are very inconspicuous in *D. septaria*, and it is probable that in certain states of contraction they are very difficult to detect. Miss Sproston, who has not herself examined *D. septaria*, refers to figures by Dollfus, Linton and Woodland. There is no evidence that the larval forms figured by Dollfus (1936, Fig. 557) belong to *D. septaria*. Dollfus calls them *D. plicitum* Linton, but from the measurements given of the bothria (640 μ long and 300 μ wide) they seem too small to be assigned with certainty to that species. Linton's figure (1922, Pl. I, fig. 1) represents the scolex of a type-specimen of *D. plicitum*, while Woodland's (1927) figures cannot, in the writer's opinion, be said to show the structures in question at all.

The dimensions of the cirrus, testes and vitellaria vary very greatly with the maturity of the segment and with preservation. No significant difference in the size of the eggs is indicated. The number of testes is not given in Sproston's description, though they are said to be 'more numerous than in *D. planum*'. An attempt was made by the writer to count them in a whole preparation of a young mature segment, and 750 was the approximate figure obtained. In *D. septaria* the number was estimated from sections by Perrenoud as 320–400. The range of variation in the number of testes is unknown, and may be very great, so that the significance of this apparent difference is doubtful.

Host: Blue Shark (*Carcharinus glaucus*).

Dinobothrium planum Linton, 1922

Synonyms:

Dinobothrium septaria of Mola, 1906.
(?) *D. septaria* of Masi, 1913.
(?) *D. septaria* of Nybelin, 1914.
(?) *D. plicitum* of Joyeux, 1923.
Phyllobothrium septaria of Southwell, 1925 (in part).
Dinobothrium planum of Guevara Pozo, 1945.
D. planum of López-Neyra, 1945.
(?) *D. plicitum* of Guevara Pozo, 1945.
(?) *D. plicitum* of López-Neyra, 1945.
D. planum of Sproston, 1948.

This synonymy differs little from that given by Sproston. Regarding the *D. septaria* of Joyeux & Baer (1936) and the *D. plicitum* of Joyeux (1923), of Guevara Pozo (1945) and of López-Neyra (1945), see the discussion under *D. plicitum*, below.

According to Linton's (1922) description 'the pseudo-hooklets at the angles' of the 'auricles' are lacking in this species, and no such processes are mentioned or figured by any of the other describers, including Mola (1906) and Masi (1913), whose material was probably *D. planum*. Nybelin's (1914) photographic figures of the scolex are too small and not sufficiently clearly reproduced to show

* Woodland gives the dimensions of the cirrus-sac as 0·99–1·32 × 0·38–0·44 mm., and Perrenoud as 1–1·4 × 0·45–0·48 mm. The writer found it, in Dr Woodland's preparations, to measure 1·1–1·8 × 0·4–0·6 mm.

† Perrenoud, however, seems to imply that at least the median processes are present, for he quotes Linton's description of *D. plicitum*, in which they are mentioned, and says: 'Cette description est précise et exacte. Elle se rapporte point par point à mes spécimens.'

such processes, if they were present, but his material was probably also *D. planum*. The writer has examined under a binocular microscope a considerable number of scolices from *Cetorhinus maximus* from different sources—Japan, Plymouth (Miss Sproston's material) and the Hebrides (three different sharks). In the larger and more relaxed scolices no processes were made out, but in those from two of the Hebrides sharks they were present, though very minute, in both outer and median positions. These were somewhat contracted scolices, showing a tendency to the formation of grooves at the posterior ends of the bothria, such as were described by Linton as characteristic of *Dinobothrium plicitum*. It seems, therefore, that the apparent presence or absence of the processes likewise depends upon the state of contraction of the scolex. In the writer's material the scolex varies considerably in size, measuring 6–9 mm. in overall length and 7–12 mm. in maximum width. In well-preserved specimens spines are present on the convex surfaces of the bothria, at least near their edges. The spines are larger and less closely set than in *D. septaria*, and appear to be readily lost if the material is not perfectly fresh.

The most important diagnostic features in the anatomy of *D. planum* are: (1) the course of the vagina, which runs transversely from the genital pore to the middle line of the segment, and then turns posteriorly; (2) the absence of vaginal sphincters; (3) the relatively small size and narrow shape of the cirrus-sac, which, in the writer's material, measures up to 1 mm. in length and not more than 0·17 mm. in width;* (4) the restriction of the vitellaria to two ventral sheets, almost entirely median to the excretory canals. The number of testes in mature segments was estimated at about 100–350, and they attain a diameter of about 0·08 mm.

Host: Basking Shark (*Cetorhinus maximus*).

Dinobothrium plicitum Linton, 1922

Synonyms:
(?) *Dinobothrium plicitum* of Joyeux, 1923.
(?) *D. plicitum* of Guevara Pozo, 1945.
(?) *D. plicitum* of López-Neyra, 1945.

The status of this species is very uncertain. Linton's (1922) original material (from *Carcharodon carcharias*) was immature, and his description contains nothing of diagnostic value concerning the anatomy of the strobila. Joyeux (1923), who, from the characters of the scolex, identified with this species four very contracted specimens from *Cetorhinus maximus* taken off Concarneau, Finistère, says that the genital organs, so far as he could determine, were 'of the same type' as in *Dinobothrium planum*. He states that the cirrus-sac is cylindrical, but does not give its measurements, and does not describe the course of the vagina. The vitellaria are said to be situated at the sides, on the ventral surface, which suggests a similar arrangement to that of *D. planum*.

Joyeux & Baer (1936) mention only one species of *Dinobothrium* (*D. septaria*), and the description given by them seems to be based on that of Perrenoud (1931). In addition to *Lamna cornubica*, however, they mention '*Selache maximus*' from Concarneau as a host. As they give a copy of Linton's figure of the scolex of *Dinobothrium plicitum* as representing that of *D. septaria*, it seems evident that they regard *D. plicitum* as a synonym of *D. septaria*.

Guevara Pozo (1945), whose material (from *Cetorhinus maximus*) was, like that of Linton, immature, was unable to add anything of importance to the description.

In view of what has been said above concerning the variability in size and shape of the scolex in different states of contraction in *Dinobothrium planum*,* the supposed differences in the scolex between *D. planum* and *D. plicitum* can hardly be considered specific. In any case, the specimens referred to by Joyeux (1923) and by Guevara Pozo (1945), from *Cetorhinus*, were in all probability *Dinobothrium planum*.

Hosts: Man-eater Shark (*Carcharodon carcharias*); [? also Basking Shark (*Cetorhinus maximus*)].

Dinobothrium paciferum Sproston, 1948

The writer has re-examined the type-material of this species, which has been kindly presented to the British Museum (Nat. Hist.) by Miss Sproston. It consists of two specimens, mounted as whole preparations on a single slide. The length of the larger specimen is given in the original description as 17·2 mm., but the writer finds it to be about 21·5 mm. The maximum width of the strobila is about 0·47 mm. The overall length of the scolex is, as Miss Sproston states, about 0·65 mm., but the width of a pair of 'auricles' is only about 0·53 mm. The writer's interpretation of the structure of the scolex differs considerably from Miss Sproston's. She states that 'the outstanding feature is the presence of two true suckers on the crest of each bothrium'—in other words, she believes that there are eight accessory suckers instead of the four (one to each bothrium) characteristic of the genus. Her figure (Fig. 20) shows seven (? eight) sucker-like structures, but on re-examination of this specimen the writer finds four real suckers, and eight muscular structures produced at their centres into small pointed processes, visible by careful focusing. These are what Miss Sproston

* Guevara Pozo (1945) gives the dimensions of the cirrus-sac as 0·6–0·7 × 0·12–0·14 mm. The writer finds that it varies, according to the age of the segment, from 0·35 to 1·0 mm. in length and from 0·1 to 0·17 mm. in width.

* See also the remarks of Joyeux & Baer (1936) under *D. septaria*.

has called 'prongs' in other species, and are situated, as usual, at the outer and median ends of the 'auricles'. Miss Sproston's figure shows two of the suckers and the bases of four (?six) of the 'prongs'. The suckers of the other side are concealed, in the preparation, by the two shown in the figure, but can be seen by deep focusing, as can the other four 'prongs'. It is not clear what the second structure from the left in the figure represents, as no such structure is now visible in that position in the preparation. The diameter of the true suckers is about 0·07 mm.

It is difficult to make out very much of the anatomy of the strobila. As Miss Sproston says, the specimens 'must have been dead for some little time when found', and they are in poor condition. A few details can, however, be added to the description. The cirrus-sac measures up to about 0·24 mm. in length and 0·086 mm. in width. Proximally it turns anteriorly, and in extended segments (as shown in Sproston, Fig. 22) sometimes almost reaches the anterior limit of the segment. Forty-two testes were counted in one segment. They attain a greatest diameter of about 0·08 mm. The vagina appears, in some of the young segments, to be lined with spines. In older segments these have either been lost or are difficult to detect. There is a loop in the vagina near its opening, either anterior or posterior to the cirrus-sac, which gives the appearance of an atrium containing a 'flange' or 'baffle-wall', as described by Sproston. The further course of the vagina has not been made out. The vitellaria are confined to two lateral bands. The writer has not been able to confirm Sproston's statement that 'in their extreme development the vitellaria extend from the lateral fields right across the proglottis on both sides'. Measurements of the eggs, in the balsam preparation, are valueless, as they are very much collapsed and shrunken.

For further discussion, see the description of the following species.

Host: Basking Shark (*Cetorhinus maximus*).

Dinobothrium spinosum n.sp.

This species, which was collected by Mr H. W. Parker from three Basking Sharks (*Cetorhinus maximus*) in the Hebrides in May 1947, is in most respects extremely similar to *Dinobothrium paciferum* Sproston. None of the numerous scolices available, however, is as small as the scolices of the type-specimens of *D. paciferum*, and the number of testes in mature segments appears to be smaller. A difference which, at first sight, appears still more striking is that in the present form the neck and the convex surfaces of the bothria are normally covered with spines. In some specimens, however, which were evidently not perfectly fresh when preserved, all or most of the spines have been lost, and it seems possible that this may have occurred in the types of *D. paciferum*, which admittedly were not fresh. There is at present no means of determining with certainty whether or not these two species are distinct, and it seems advisable to treat them as such until further evidence is available.

Well-preserved, moderately extended specimens of *D. spinosum*, when mounted in Canada balsam, measure up to 30 mm. in length, and the strobila has a maximum width of 0·74–0·9 mm. There is a long, unsegmented neck which, in such specimens, measures 7–11 mm. in length and varies in width from 0·1 to 0·25 mm. anteriorly, and from 0·3 to 0·5 mm. posteriorly, according to the degree of extension or contraction. In abnormally extended specimens the neck may attain a length of as much as 28 mm., and is then correspondingly reduced in width. Behind the neck there is usually a rather rapid increase in the width of the strobila. The neck is covered throughout with flattened cuticular spines (Figs. 1, 5), arranged in more or less regular alternating transverse rows. The spines cease abruptly where segmentation begins, and appear to be easily lost in specimens which have been slightly macerated or were not perfectly fresh when preserved.

The scolex (Fig. 1) is of the same general form as in other species of the genus. It varies considerably in size, but this is, of course, at least partly to be accounted for by differences in state of contraction. The four large bothria, in the relaxed condition, are elongate, shallow and tongue-shaped. On their edges and convex surfaces they are armed with numerous spines, those on the edges being extremely small, but passing over on the convex surfaces to spines more closely resembling those of the neck in size and shape. The overall length of the scolex, from the apex to the free ends of the bothria, varies between 1 and 2·2 mm. Its width anteriorly (i.e. the width of the pair of ridge-like thickenings or 'auricles' corresponding to each pair of bothria) varies between about 0·7 and 1·2 mm. Each of these thickenings is produced at its outer and median angles into prominent, sharply pointed, hook-like, conical processes (Figs. 1, 2, *mp*, *op*). These are highly muscular, being provided with powerful bands of circular muscle-fibres. In close apposition to the median process of each bothrium, and on its outer side, facing obliquely outwards, there is a circular muscular sucker (Figs. 1–3, *su*) measuring about 0·14–0·18 mm. in outside diameter. These are true suckers, with a distinct type of musculature. On the anterior lip of each there are three or four rows of small, spine-like tubercles. The outer or lateral border of each bothrial ridge is produced, as in other species, into a flattened flange (Fig. 1, *f*) with its free end deeply notched.

The bothria are continuous only anteriorly with the neck. Farther back they are joined for some

distance by membranes (Figs. 1, 4, *m*) which surround the neck but are not connected with it. The bothria are abundantly supplied with stout bundles of muscle-fibres vertical to their broad surfaces, and the ends of these bundles produce a reticulated appearance in cleared specimens in surface view. Muscle-bundles of a similar nature extend into the bases of the conical processes (Fig. 3, *mp*, *op*).

The strobila consists of about 50–60 segments, of which the last 10–20 are gravid. These are preceded by only five or six segments in which the genital organs appear to be at about their full development, while all the rest are immature. The young segments are broader than long, and mature segments square or slightly longer than broad, while gravid segments increase in length until they may be more than twice as long as broad. The strobila is relatively thick dorso-ventrally (Figs. 6, 7). The genital pores are irregularly alternating and situated at about the middle of the lateral border of the segment (slightly behind the middle in gravid segments). The cirrus-sac and vagina open side by side into a small common atrium, but the cirrus-sac may be either anterior or posterior to the vagina in different segments of the same strobila. At its inner end, however, it is always dorsal, and the vagina runs inwards ventrally to it.

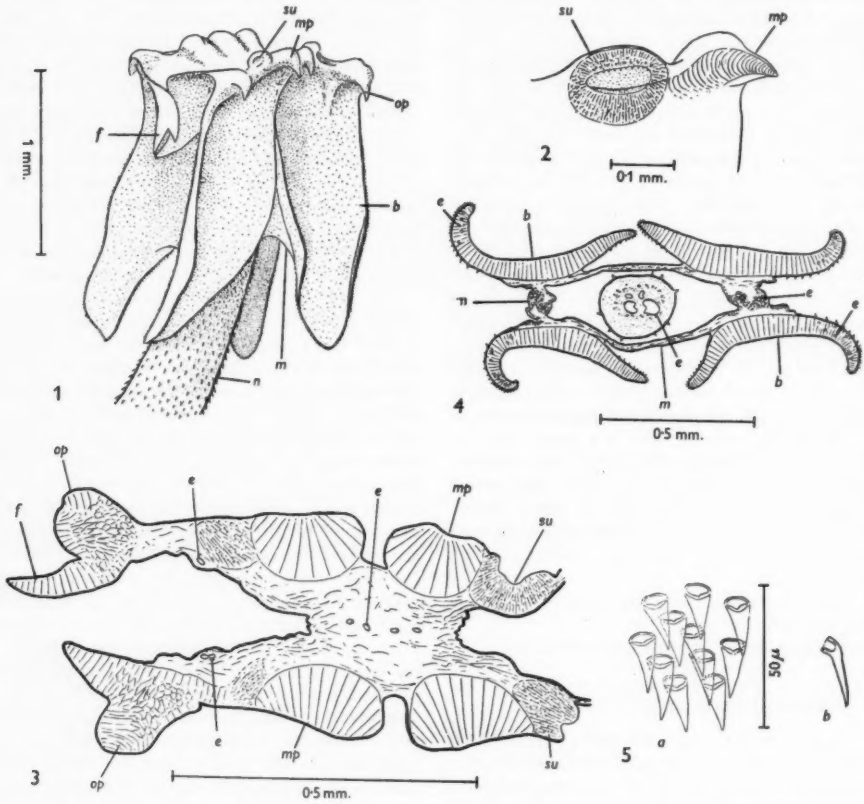


Fig. 1. *Dinobothrium spinosum*. Scolex, viewed as an opaque object, without pressure (obliquely dorso-ventral view). *b*, bothrium; *f*, flange; *m*, membrane joining bothria; *mp*, median process; *n*, neck; *op*, outer process; *su*, sucker.

Fig. 2. *Dinobothrium spinosum*. Median process and sucker (oblique view), from a whole preparation. *mp*, median process; *su*, sucker.

Fig. 3. *Dinobothrium spinosum*. Transverse section through anterior part of scolex. *e*, branch of excretory canal; *f*, flange; *mp*, median process; *op*, outer process; *su*, sucker.

Fig. 4. *Dinobothrium spinosum*. Transverse section through neck and bothria. *b*, bothrium; *e*, branch of excretory canal; *m*, membrane joining bothria.

Fig. 5. *Dinobothrium spinosum*. Spines on neck. *a*, a group seen from above. *b*, a single spine in profile.

Both cirrus-sac and vagina pass between the dorsal and ventral excretory canals, and ventrally to the nerve-cord, of the pore side.

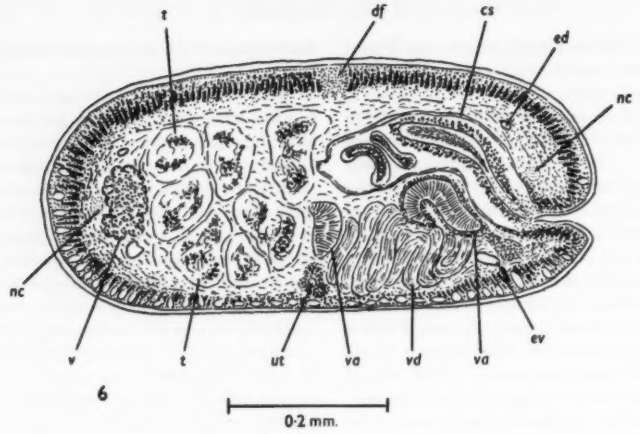
The subcuticular layer is of considerable depth in the segmented portion of the strobila, and composed largely of gland-cells. Many of these contain a large clear space which appears to open by a pore in the cuticle. The subcuticular layer is considerably thinner on the ventral surface than elsewhere (Figs. 6, 7). The musculature of the strobila is relatively feebly developed, and seems to consist almost entirely of longitudinal fibres. These are abundant in the neck region, but much less conspicuous in the segmented portion. The usual thin layers of circular and longitudinal subcuticular fibres are present. The rest of the longitudinal fibres do not form regular bundles, but are interspersed in vertical series, sometimes forming slight aggregations, between the cells of the subcuticula. In the mid-dorsal line, however, there is a conspicuous aggregation of longitudinal fibres forming a strong dorsal fascia (Figs. 6, 7, *df*). In the mid-ventral region the musculature is scanty. The absence of a deeper layer of transverse muscle-fibres seems to be characteristic of the genus. Linton (1922) failed to find such a layer in *D. planum* or *D. plicitum*, and Woodland (1927) also failed to find it in *D. septaria*. There is thus, as Woodland observes, no visible boundary between the cortical and medullary regions of the parenchyme.

There are the usual two pairs of longitudinal excretory canals, the dorsal being narrower than the ventral. They have no transverse connexions, and no intersegmental openings to the exterior, such as have been described by Woodland (1927) for *D. septaria*, but not confirmed by Perrenoud (1931). In the scolex the canals give off branches which run down the interbothrial membranes and appear to form networks in the outer edges of the bothria (Figs. 3, 4, *e*).

The cirrus-sac (Figs. 6, 8, 9, *cs*) is large, but its wall is thin and feebly supplied with muscles. It measures about 0·4 mm. in length and 0·15 mm. in greatest width when fully developed. It curves forward at its inner end, and extends to the middle line of the segment or beyond it. The cirrus is long and densely covered with spines. For part of its length it has a coat of gland-cells. There is no internal or external seminal vesicle, but the vas deferens, which forms a large mass of coils ventrally and anteriorly to the cirrus-sac, provides ample storage for sperm. Each mature segment contains 23–30 testes which, when fully developed, have a maximum diameter of about 0·1 mm. They occupy at first most of the space in the medullary portion of the segment, where it is not occupied by the ovary, vitellaria and genital ducts, and may then in places form two layers. Later, most of them are pushed dorsally and laterally by the development of theuterus, and then form a single layer.

Near its opening the vagina (Figs. 6, 8, 9, *va*) is a wide duct with a dense external coat of deeply staining cells, but apparently without sphincters. It usually forms a conspicuous loop, directed anteriorly or posteriorly according to its position relative to the cirrus-sac, and often one or two dorso-ventral loops (Fig. 9). In this region its thick cuticular lining appears to break up into bristles. Soon, however, the character of the walls of the tube changes, and it takes a course almost straight across the segment, ventrally to the cirrus-sac, until it nearly reaches the excretory canals of the opposite side. Here it turns at right angles and runs posteriorly, with a sinuous course, finally turning inwards again, now as a thin-walled and still much convoluted tube, to connect with the gland-complex in the neighbourhood of the ovarian isthmus. A portion of it just in front of this isthmus (Figs. 7, 8, *rs*) expands to form a receptaculum seminis. The ovary (Figs. 7, 8, *ov*) is situated at the posterior end of the segment, and is much compressed antero-posteriorly. In transverse section (Fig. 7) it is not so obviously X-shaped as in many Tetraphyllidea, but forms a double fan of large lobes joined by a transverse isthmus. The lobes occupy the whole dorso-ventral depth of the medullary region, and extend laterally as far as the vitellaria. The latter form two lateral longitudinal columns of follicles (Figs. 6–8, *v*) between the dorsal and ventral excretory vessels and just internally to the nerves, and extend in almost unbroken series from segment to segment, being interrupted only on the pore side of each segment by the genital ducts. The shell-gland (Mehlis' gland) lies immediately behind and dorsally to the ovarian isthmus, at the extreme posterior limit of the segment, and has a diameter of about 0·07 mm.

The uterus first appears as an almost solid column of cells running longitudinally along the ventral side of the segment, almost from end to end, just within the subcuticular layer. In transverse section (Figs. 6, 7, *ut*) it is seen to have a very narrow lumen. Later, as it becomes filled with eggs, it expands laterally and dorsally, forming two main thin-walled sacs, anterior and posterior to the transverse limb of the vagina, which becomes enveloped by them. There appears to be no preformed uterine opening to the exterior, and the eggs are probably liberated by dehiscence of the wall of the segment ventrally, where, as already stated, it is thinner than elsewhere. The eggs are small and spherical. The outer envelope, after restoration of its natural contour by treatment with lactophenol, has a diameter of 36–38 μ. The onchosphere, which is closely invested by the inner membrane, measures about 16 μ in diameter, and the embryonic hooks are about 7 μ long.



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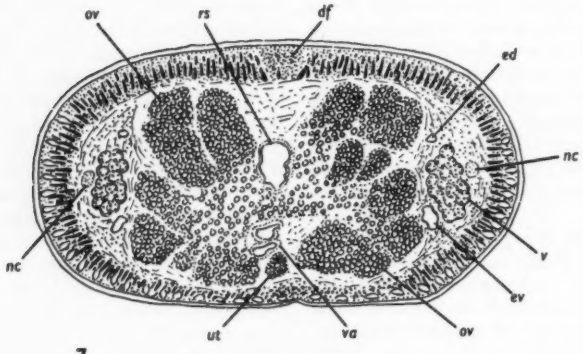

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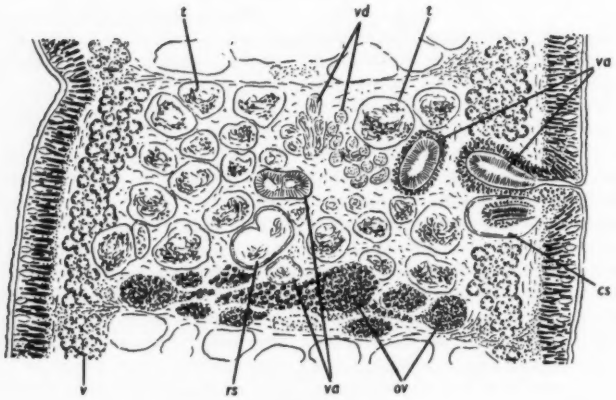

8

0·2 mm.

Figs. 6–8

LARVAL FORMS

Linton (1897) recorded, under the name of *Thysanocephalum* sp. ('presumably of the species *T. crispum*') a larval form from the stomach of the squid *Ommastrephes illecebrosus* at Woods Hole, Massachusetts. This 'scolex' measured 1 mm. in length, and appears from the figures, as Sproston points out, to be referable to *Dinobothrium*, and not to *Thysanocephalum*. Dollfus (1936) describes larvae which he considers to belong to the same species as Linton's, from the intestinal wall of another squid, *Todaropsis eblanae*, off Finistère, Brittany. His specimens measured 0·7–1·2 mm. in length and 0·55–0·7 mm. in width, and the bothria measured up to 0·64 mm. in length and 0·3 mm. in width.

Dollfus refers these larval forms to *Dinobothrium plicitum*, and Sproston to *D. septaria*. It is not yet known to what extent the scolex of *Dinobothrium* may grow after being introduced into the alimentary canal of the final host. The considerable variation in size observed in scolices of the same species has been mentioned in discussing *D. planum* and *D. spinosum*. Sproston states that the largest scolex of *D. keilini* measured, before fixation, over 8 mm. long and 10 mm. wide. This scolex, now in Canada balsam, measures about 7 mm. in length and 9 mm. in width. Together with this specimen there were two 'young' scolices. In the smaller of these the pairs of bothria, according to Sproston, measured only about 1·12 mm. long and 1·6 mm. wide, while in the other they measured 2·15 mm. long and 2·27 mm. wide. The overall dimensions of the latter scolex, now mounted in balsam, are about 2·5 × 3 mm. These examples seem to afford evidence that a good deal of growth may take place in the scolex in the final host, but no larval form is yet known with a scolex of anything like the size of that of the adult *D. planum*, *D. plicitum*, *D. septaria* or *D. keilini*. The larvae described by Linton and by Dollfus seem more comparable with the adult scolices of *D. paciferum* or *D. spinosum*, but both these species occur in the Basking Shark, which feeds on small planktonic organisms, and it is doubtful whether it would be capable of swallowing squids, unless they were extremely small.

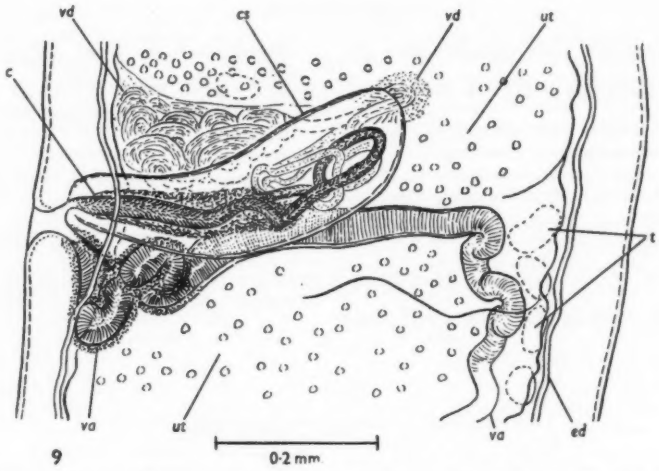

Fig. 9. *Dinobothrium spinosum*. Middle portion of a gravid segment; dorsal view (from a whole preparation). *c*, cirrus; *cs*, cirrus-sac; *ed*, dorsal excretory canal; *t*, remains of testes; *ut*, cavity of uterus; *va*, vagina; *vd*, vas deferens.

Legends to figs. 6–8

Fig. 6. *Dinobothrium spinosum*. Transverse section through the anterior portion of a mature segment. *cs*, cirrus sac; *df*, dorsal fascia; *ed*, dorsal excretory canal; *ev*, ventral excretory canal; *nc*, nerve-cord; *t*, testis; *ut*, uterus; *v*, vitellaria; *va*, vagina; *vd*, vas deferens.

Fig. 7. *Dinobothrium spinosum*. Transverse section through the posterior portion of a mature segment. *df*, dorsal fascia; *ed*, dorsal excretory canal; *ev*, ventral excretory canal; *nc*, nerve-cord; *ov*, lobe of ovary; *rs*, receptaculum seminis; *ut*, uterus; *v*, vitellaria; *va*, vagina.

Fig. 8. *Dinobothrium spinosum*. Horizontal section through a mature segment. *cs*, cirrus-sac; *ov*, ovary; *rs*, receptaculum seminis; *t*, testis; *v*, vitellaria; *va*, vagina; *vd*, vas deferens.

SUMMARY

A critical review is presented of the species of *Dinobothrium* and their probable synonyms. *D. septaria* v. Beneden and *D. planum* Linton are accepted as valid species. *D. keilini* Sproston and *D. plicitum* Linton are regarded as of doubtful status, the former being probably a synonym of *D. septaria*, while *D. plicitum* may be a synonym of *D. planum*. The type-specimens of *D. keilini* and of *D. paciferum* Sproston have been re-examined. A new interpretation is offered of the structure of the scolex of *D. paciferum*, and some other details of its anatomy are redescribed. A small species from *Cetorhinus maximus* is described, which is very similar to, and may prove to be identical with, *Dinobothrium paciferum*. As it apparently differs in having the neck and parts of the bothria covered with spines, it is treated as a distinct species and named *D. spinosum*. The question of the specific identity of the known larval forms of *Dinobothrium* is briefly discussed.

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THE VARIATION OF THE BRITISH HARVEST MITE (TROMBICULIDAE, ACARINA)

By W. S. RICHARDS, M.A., D.Phil., F.R.E.S.

(With 23 Figures in the text)

The British harvest mite is well known to those unfortunate enough to suffer from its attacks, which are distressing rather than dangerous. Related species, though producing no irritation, transmit 'scrub typhus', which proved a major cause of morbidity during the late campaigns in South-East Asia. As these species are similar to our own in their habits and mode of life, methods of studying them can be developed in this country under more favourable conditions than can be found in a humid tropical area.

By way of preliminary, the external anatomy of a large number of British specimens was examined and compared, since differences in morphology where present are often an excellent indication of corresponding differences in behaviour and mode of life.

There are four stages in the life history—egg, larva, nymph and adult. The eggs are white and spherical, about 0·2 mm. in diameter. The six-legged orange larvae first appear in July and reach their maximum by the beginning of September. Very few remain after the first frosts though stragglers may still be present the following spring. Unfed larvae are most active in dry sunny weather. If they find a suitable host (and they have been recorded from most British mammals and some birds, e.g. partridge), they may take only one day to engorge, feeding not on blood but on a localized area of disintegrating epidermis or histiosiphon induced by the bite (André, 1930). During the period each swells from 0·25 to 0·75 mm. in length, and turns pale yellow. When fully fed, they leave their host, crawl down into the soil and remain quiescent while the eight-legged nymph develops within the larval skin. The dense covering of white plumed hairs and the constricted waist distinguishes the nymph from all other British mites. Like the South-East Asian vectors of scrub-typhus, it is subterranean and predaceous.

The adult differs from the nymph mainly in size, 2 mm. as against 1 mm. for the nymph, and in the possession of sexual character. Like the nymph they live entirely below ground.

The different stages required different techniques for their collection. Samples of the ears of wild rabbits were sent to me by post from various parts of the country. The abundance and external anatomy of the larvae feeding on these ears proved an excellent guide to the distribution and variation of the harvest mite in Great Britain. The 'preattachment' larvae present in a single square yard of ground were collected by placing a cover over the area. This had a central hole capped by a glass collecting tube. Larvae trapped under the cover crawled towards the daylight shining through the central hole and were caught in the glass tube (the principle of this method was devised by my technical assistant, Mr K. L. Cockings). Nymphs were reared from gorged larvae. Adults were extracted from the soil by flotation in water. These methods are described in detail by Cockings (1948).

The material examined in the course of this investigation included: the collections in the British Museum of Natural History, those possessed by Mr C. Elton and Mr F. A. Turk, and my own collection of 300 samples of larvae, twenty-five samples of nymphs and three samples of adults. Of the samples of larvae, 250 came from England, eighty from Wales, twenty-two from Scotland (including the isles of Colonsay, Coll, Eigg and Gometra) and three from Ireland.

The variation of the larvae, nymphs, and adults is described in the following pages and its bearing on speciation is discussed.

LARVAL VARIATION

As the majority of Trombiculid adults remain unknown, the taxonomy of this group is based on larval systematics.

Among the more important characters used to classify Trombiculid larvae are the nature of the chelicerae, the number of palpal claws, the chaetotaxy of the galeae and the fourth (tibial) palpal segment, the number of tarsal claws, the arrangement and number of long simple sensory setae on the tarsus of the third pair of legs, the number of coxal setae, the number and arrangements of the dorsal scutes, the arrangement and shape of the scutal setae, and the number and arrangement of the postscutal dorsal setae.

All the British specimens so far observed have the following characters in common: chelicerae with two small apical teeth, one external lateral and one ventral; three palpal claws, three tarsal claws, a single long sensory hair on the tarsus of the third pair of legs, one seta per coxa; a single pentagonal scutum bearing marginal setae antero-medianly and at the two lateral angles, and a central pair of filamentous ciliated sensillae; and twenty-five to fifty post-scutal dorsal setae arranged in transverse rows. The members of the first row are called humeral setae and are usually few.

The British specimens can be further grouped into those with galeal, dorsal and lateral tibial palpal setae ciliated (Fig. 1), and into those with these setae simple (Fig. 2). The latter can be differentiated on the nature of the scutal chaetotaxy into those with a single seta at each of the four lateral angles and a single antero-median seta, and into those in which one or more of these setae are doubled (usually the postero-lateral scutal setae) (Fig. 4). The individuals with single marginal scutal setae can again be divided into those with four humeral setae (Fig. 3) and into those with two. Most British specimens belong to the last type which have simple galeal and dorsal and lateral tibial palpal setae, single scutal setae and single humeral setae. Henceforth they will be referred to as the normal type.

These 'normal' forms can be further separated into three classes based on the number and arrangement of the post-humeral dorsal setae. Normal type *a* has eight or more setae in both the second and third dorsal rows; normal type *b* has six in each; normal type *c* has eight setae in the second row but only six in the third. Normal type *a* appears to be associated with a dorsal setal count in the region of thirty-six, and type *b* with a count in the region of thirty but the number of setae in each row is variable and the setal rows become confused towards the posterior region of the body at which point the dorsal setae pass into the ventral setae and the precise number of each is difficult to determine.

In addition to the characters described above, Womersley & Heaslip (1943) have devised a comprehensive scheme for separating species of *Trombicula* using a biometric system to discriminate between species. To simplify comparison, I have considered two measurements only: the width of the scutum between the bases of the antero-lateral scutal setae (*AW*) and the length of the postero-lateral scutal setae (*PL*), the former exemplifying scutal dimensions and the latter, setal measurements.

Fig. 7 illustrates the association of these characters with the main types of larval variation.

This figure is based on an examination of six samples of larvae from five different localities. They were selected to show the type of conclusions which can be drawn from a limited range of material. Study of the figure shows that forms with ciliated galeal setae are characterized by long postero-lateral scutal setae, six to eight setae in the second row of dorsal setae, and six in the third. With 'duplicated postero-lateral scutal setae' are associated short postero-lateral scutal setae and a large number of dorsal setae. Duplicated humeral setae are similarly correlated with a large number of dorsal setae, but the postero-lateral scutal setae are long, not short.

Normal larvae were sorted into those with eight or more setae in both the second and third dorsal rows (type *a*, Fig. 5), those with six setae in both (type *b*, Fig. 2) and into those with eight in the second but only six in the third (type *c*). In each sample of larvae the width of the scutum and the length of the postero-lateral scutal setae were plotted for all individuals with the same type of setal count.

In larvae with eight or more setae in both the second and third dorsal rows (type *a*), those from Eigg, Hebrides, tended to have a greater width of scutum and longer postero-lateral scutal setae than had larvae collected at Warren Farm, near Streatley, Berkshire. The Warren Farm samples also contained type *b* larvae with few dorsal setae. Apart from the dorsal setal count, they resembled the type *a* larvae of the same date in width of scutum and length of postero-lateral scutal setae. Larvae collected in the same locality during July, though similar in setal count to the type *b* August and October larvae, were wider in the scutum. This width of scutum was shared by type *b* larvae from Lydham, Salop, but the July Warren Farm larvae differed from them in the shortness of their postero-lateral scutal setae.

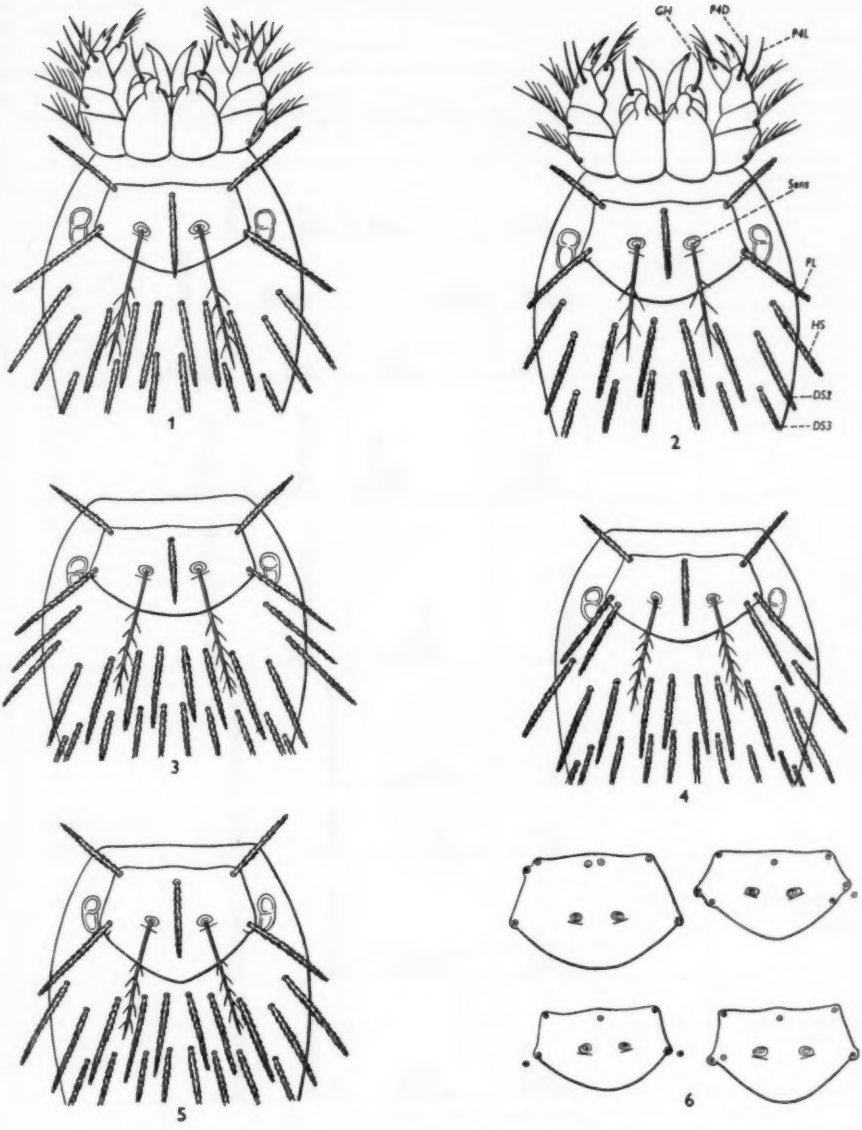
The last sample is from Dorset. Of the first twenty larvae examined, fifteen were typical type *c*, four type *b* and one had seven setae in the second dorsal row and eight in the third. Scutal widths and setal lengths did not fall outside the values recorded for normal type *a* and type *b* larvae.

In this way, the larvae derived from five localities can be separated into nine different types—each of which has a characteristic scutal width, postero-lateral scutal setal length and post-humeral dorsal setal number. Three of these types are still further distinguished by ciliated galeal setae, duplicated postero-lateral scutal setae and duplicated humeral setae.

The characters determining these types will now be considered in the light of my remaining material to determine whether the differences observed are due to the influence of local conditions on larval development or whether they can be attributed to genetical factors which might justify their description as separate races or species.

For example, ciliated galeal setae are associated with ciliated dorsal and lateral tibial palpal setae, an antero-median scutal seta sufficiently long to project beyond the hind edge of the scutum, long postero-

Fig. 1
Fig. 2
=
=
Fig. 3
Fig. 4
Fig. 5
Fig. 6



Figs. 1–6. The main types of larval variation.

Fig. 1. Galeal hairs cileated.
Fig. 2. Normal type *b*. *GH* = galeal hair; *P4D* = dorsal tibial palpal hair; *P4L* = lateral tibial palpal hair; Sens = sensillae; *PL* = postero-lateral scutal setae; *HS* = humeral setae; *DS2* = setae of second dorsal row; *DS3* = setae of third dorsal row.
Fig. 3. Duplicated humeral setae.
Fig. 4. Duplicated postero-lateral scutal setae.
Fig. 5. Normal type *b*.
Fig. 6. Some scutal aberrations.

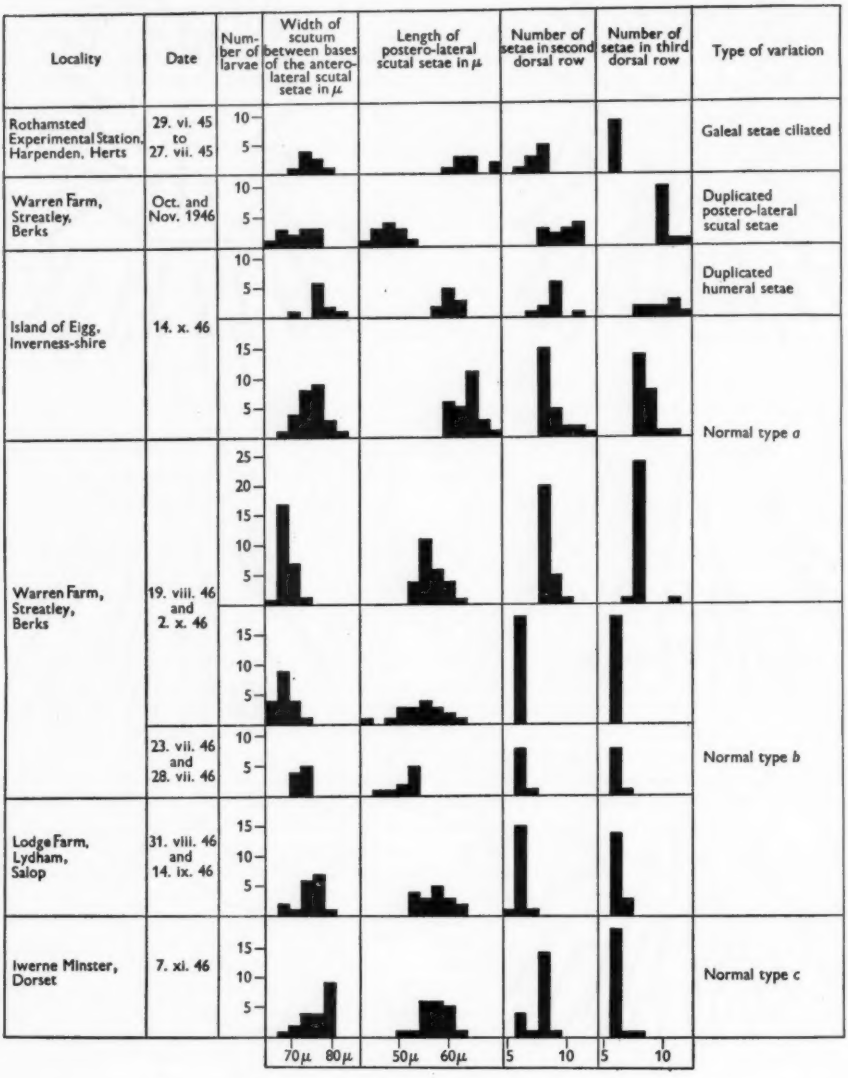

Locality
Date
Number of larvae
Width of scutum between bases of the antero-lateral scutal setae in μ
Length of postero-lateral scutal setae in μ
Number of setae in second dorsal row
Number of setae in third dorsal row
Type of variation
Rothamsted Experimental Station, Harpenden, Herts
29. vi. 45 to 27. vii. 45
Galeal setae ciliated
Warren Farm, Streatley, Berks
Oct. and Nov. 1946
Duplicated postero-lateral scutal setae
Duplicated humeral setae
Island of Eigg, Inverness-shire
14. x. 46
Normal type a
Warren Farm, Streatley, Berks
19. viii. 46 and 2. x. 46
23. vii. 46 and 28. vii. 46
Normal type b
Lodge Farm, Lydham, Salop
31. viii. 46 and 14. ix. 46
Iwerne Minster, Dorset
7. xi. 46
Normal type c
70μ 80μ
50μ 60μ
5 10
5 10

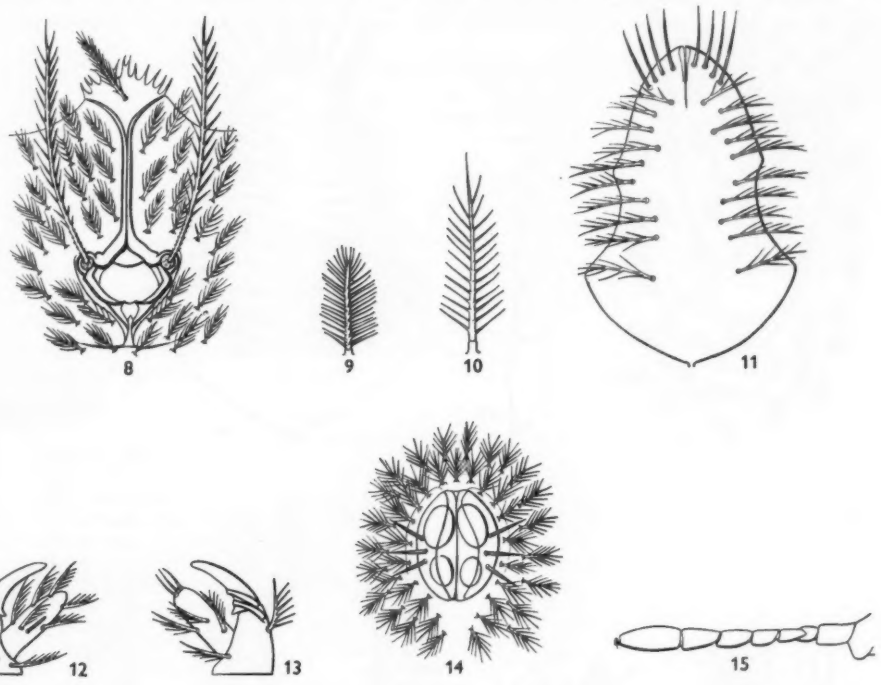
Fig. 7. Analysis of variation. The scutal widths, setal lengths, and setal counts associated with the five main types of larval variation.

lateral scutal setae, and seven to eight setae in the second dorsal row.

However, long antero-median and postero-lateral scutal setae are not restricted to larvae with ciliated galeal setae, but exist in a small proportion of the normal larvae found in the same samples. Both simple and ciliated galeal setae and dorsal and lateral tibial palpal setae occur in the same individual. These intermediate forms tend to resemble the normal type *b* in setal measurements and setal feature in gross appearance or behaviour tends to confirm this view.

Similarly, although in the samples listed in Fig. 7, short postero-lateral scutal setae and a large number of post-humeral dorsal setae were associated with duplicated postero-lateral scutal setae; these characters are variable and do not always occur together.

Thus the duplication of postero-lateral scutal setae is not always symmetrical. Many individuals have setae duplicated on one side only, one has two



Figs. 8–15. Nymphal characters of systematic importance.

Fig. 8. Crista metopica. × 250.
Fig. 9. Posterior hair, type *a*. × 250.
Fig. 10. Posterior hair, type *b*. × 250.
Fig. 11. Hypostome. × 250.
Fig. 12. Tibia and tarsus of palp, outer face. × 250.
Fig. 13. Tibia and tarsus of palp, inner face. × 250.
Fig. 14. Genitalia. × 250.
Fig. 15. Right first leg. × 50.

counts. In addition, the number of setae in the second dorsal row varies between six and ten and two larvae lack a scutal seta.

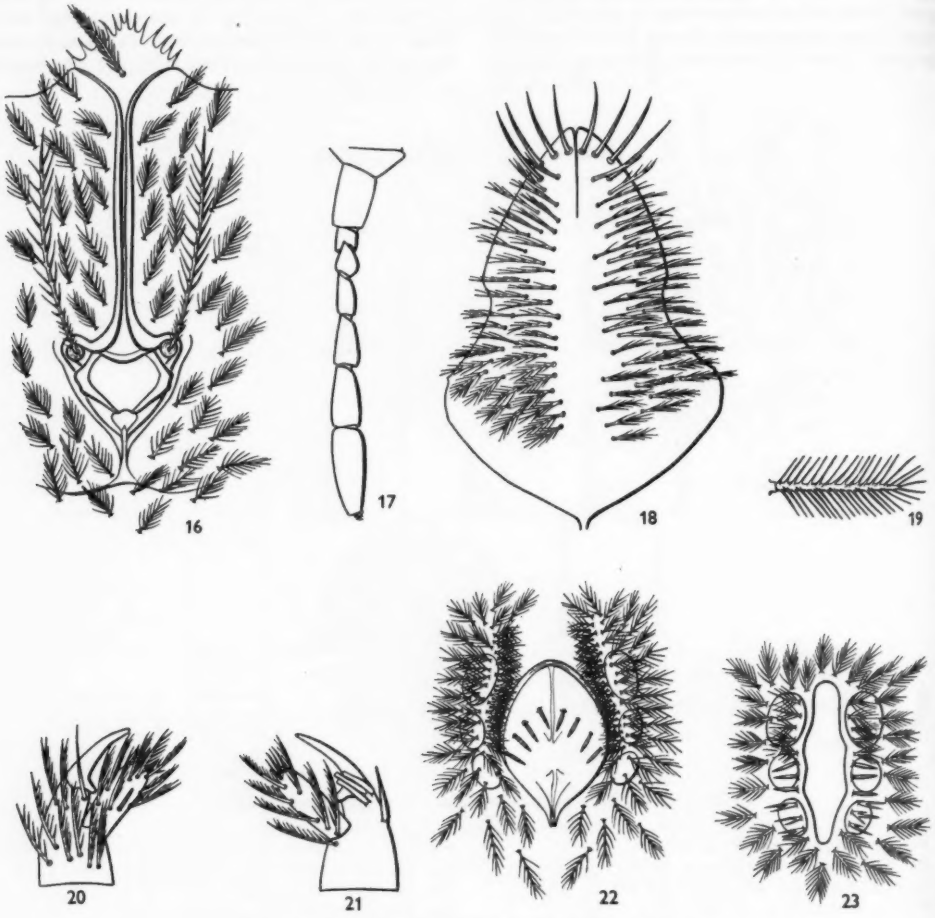
This inconsistency in the expression of characters associated with ciliated galeal setae suggests that these deviations from the normal type *b* are not genetically stable but due to the effect of some external agent upon the course of development of individual larvae. The sporadic distribution of this type (Table 1) and the absence of any distinguishing

on the left and three on the right (Fig. 6); and the duplicated seta does not always lie inside the scutal area (Fig. 6). Duplicated postero-lateral scutal setae are not always short, or associated with a large number of post-humeral dorsal setae. Three individuals are known with a type *b* dorsal setal count.

Even in the sample cited above, the range of postero-lateral scutal setal measurements and of dorsal setal number does not lie outside that for normal type individuals. As for other variations in

the number of peripheral scutal setae, one specimen lacks an antero-median scutal seta, one a postero-lateral scutal seta, while two have duplicated antero-lateral scutal setae (on one side only) and one of these has the antero-median scutal setae also duplicated (Fig. 6).

greater constancy in their association with moderately long postero-lateral scutal setae and a high dorsal setal number. Such lack of variation suggests that this form may be genetically distinct. However, these last two characters lie within the range observed in normal individuals and no difference can



Figs. 16–23. Adult characters of systematic importance.

Fig. 16. Crista metopica. × 250.
Fig. 17. Right first leg. × 50.
Fig. 18. Hypostome. × 250.
Fig. 19. Posterior hair. × 250.
Fig. 20. Tibia and tarsus of palp, outer face. × 250.
Fig. 21. Tibia and tarsus of palp, inner face. × 250.
Fig. 22. Male genitalia. × 250.
Fig. 23. Female genitalia. × 250.

This variability suggests that these scutal aberrations are to be regarded as phenotypic variations of the normal course of development, rather than racial or specific differences.

Duplicated humeral setae show a considerably

be observed in general appearance or biology. Therefore the genotypic nature of this variation must be regarded as unproved.

Normal type forms themselves appear divisible into those with eight or more setae in the second and

third dorsal rows, type *a*, and into those with six, type *b* (Fig. 7). If wild populations from different areas or from the same area but at different times of year be compared, the mean values of scutal width and postero-lateral scutal setal length may differ though the number of dorsal setae be the same. Moreover, samples may consist almost entirely of larvae with eight setae in the second row but only six in the third (normal type *c*), being intermediate between normal type *a* and normal type *b* (Fig. 7).

To investigate the relation between these three types of measurement, namely, scutal width, setal length and setal number, each of these characters was plotted in turn against the other two (Table 3). No correlation was observed between any pair of these characters, although each character could be correlated with similar types of measurement. So, although the width of the scutum between the bases of the antero-lateral scutal setae is correlated with the width between the base of the postero-lateral scutal setae, the length of the postero-lateral scutal setae with the length of the humeral setae, and the number of setae in the second dorsal row with the number of barbs on the sensillae, scutal width is not correlated with setal length or setal number, and setal number is not correlated with setal length.

Therefore, if harvest mites be separated into two groups on the basis of dorsal setal number, then each type has the same range of scutal width and setal length. Moreover, specimens collected on 19 August 1946 and 2 October 1946 on the Fair Mile near Streatley, Berkshire, though divisible into two types on the basis of the number of dorsal setae, yet shared a common range of scutal width and setal lengths. This population differed in these two characters from other wild populations collected either at different times in the same area or in different areas at the same time (Fig. 7). In other words, the factors governing scutal width and setal length, while common to both types of setal count, appear to vary independently of those factors which govern the number of dorsal setae. This suggests that in these characters, at least, both types of dorsal setal count share a common heredity.

So far as the number of dorsal setae themselves are concerned, their number tends to be still further increased in forms with an increased number of humeral or dorsal setae. Furthermore, forms occur (type *c*) intermediate in setal count between type *a* and type *b* larvae. This suggests that setal number is in part a plastic character (Fig. 7). If such be the case, then the gap between individuals with six

Table 1. *Proportion of abnormal types in those samples in which they occur*

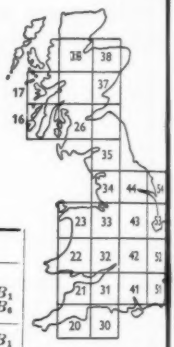
			No. of normal types in sample		No. of abnormal types in sample		
Date	Locality	Remarks	*DS* 2 with 6 or 7 setae	*DS* 2 with 8 or more setae	Galeal hairs forked	Duplicate humeral setae	Duplicate postero-lateral scutal setae
28. viii. 26	Bagley Wood, Berks	*Microtus*	1	—	1	—	—
9. xi. 27	,,	*Apodemus*	—	—	1	—	—
5–7. xii. 27	,,	*Clethrionomys*	1	1	1	—	—
8. viii. 28	,,	*Apodemus*	—	—	1	—	—
12–13. viii. 28	,,	*Clethrionomys*	1	—	1	—	—
29. vi. 45	Rothamsted, Herts	*Microtus*	2	—	1	—	—
30. vi. 45	,,	,,	3	—	1	—	—
4. vii. 45	,,	*Apodemus*	2	—	6	—	—
27. vii. 45	,,	Bare earth	5	—	3	—	—
3. viii. 45	Aldbury, Herts	,,	11	—	1	—	—
10. x. 27	Bagley Wood, Berks		1	—	—	1	—
14. x. 46	Eigg, Hebrides	Rabbit	—	26	—	10	—
17. x. 46	Reskadinnik, Cornwall	,,	2	3	—	18	1
18. x. 46	Grampound, Cornwall	,,	7	—	—	1	—
26. x. 46	Pembridge, Hereford	,,	—	8	—	2	—
28. x. 46	Cricceith, Caernarvon	,,	—	—	—	1	—
2. xi. 46	Shepton Mallet, Somerset	,,	6	—	—	2	—
12. xi. 46	Marlborough, Wiltshire	,,	—	—	—	1	—
24. viii. 46	Cilmaenllwyd, Carm.	,,	1	—	—	—	1
20. x. 46	Streatley, Berks	,,	9	1	—	—	8
29. x. 46	,,	Open ground	1	—	—	—	6
1. xi. 46	Market Harborough, Leics	Rabbit	8	—	—	—	21
4. xi. 46	Blandford, Dorset	,,	4	8	—	—	4

setae in the second dorsal row and those with eight or more, may be explained on the hypothesis that the external factor influencing increase in number of dorsal setae, acts at first in a symmetrical manner by adding one extra seta to each half of each dorsal row. When these conditions are accentuated, the symmetry of the developing larvae breaks down and of characters found in larvae from the same locality is due to their having developed in the same environment.

If harvest mite populations be distinguished on setal length, instead of setal number, then this character will cut across classifications based on setal number or on scutal width. Similarly, if

Table 2. *The influence of geographical position, altitude and time of year on the ciliation of harvest-mite larvae*

100 km. map ref.		16, 26, 17			37, 28, 38				44								
8. viii. to 1. ix. 1946		A_3	—	—	A_5	—		—									
22. x. to 15. xi. 1946		A_3	—	—	A_3	—		—									
100 km. map ref.					34, 35				44								
8. viii. to 1. ix. 1946	Ht. in ft. 600				—	—		—	—	AB_1	—						
	400				—	—		—	—	—	—						
	200				—	—		—	A_5	ABX_1	B_2						
22. x. to 15. xi. 1946					A_1	A_2		—	A_2	—	—						
100 km. map ref.		23			33				43				53				
8. viii. to 1. ix. 1946	Ht. in ft. 400	—	—	—	A_6	—		—	A_3	—	—		—	—	B_1		
	200	A_3	—	—	—	—		—	—	—	B_1		—	—	B_6		
22. x. to 15. xi. 1946		A_1	AB_1	Y_1	A_1	—		—	—	—	—		—	—	B_1		
100 km. map ref.		22			32				42				52			62	
8. viii. to 1. ix. 1946	Ht. in ft. 1500	AZ_1	—	—	—	—		—	—	—	—		—	—	—	—	—
	600	BX_1	AB_1	B_1	—	—	B_2		—	—	B_4		—	—	—	—	—
	400	BY_1	AB_1	B_3	—	—		—	—	—	—		—	—	B_5	—	—
	200	—	AB_1	B_6	—	—		—	—	—	—		—	—	B_6	—	B_2
22. x. to 1. ix. 1946		—	AY_1	B_1	A_2	AC_1	AY_1		A_1	BX_1	—		—	—	B_1	—	—
100 km. map ref.		21			31				41				51				
8. viii. to 1. ix. 1946	Ht. in ft. 600	—	—	—	—	—		—	—	—	B_1		—	—	—		
	400	—	—	B_1	—	—	B_2		—	—	B_7		—	—	B_2		
	200	—	—	—	—	—	—	—	—	—	B_1		—	BC_1	—		
22. x. to 15. xi. 1946		—	—	—	C_1	CX_1	BY_1	B_1	A_1	ABX_1	Y_1	AB_1	A_1	—	—		
100 km. map ref.		20			30												
8. viii. to 1. ix. 1946		—	—	B_1	—	—	B_2										
22. x. to 15. xi. 1946		AC_2	—	—	—	—	—										



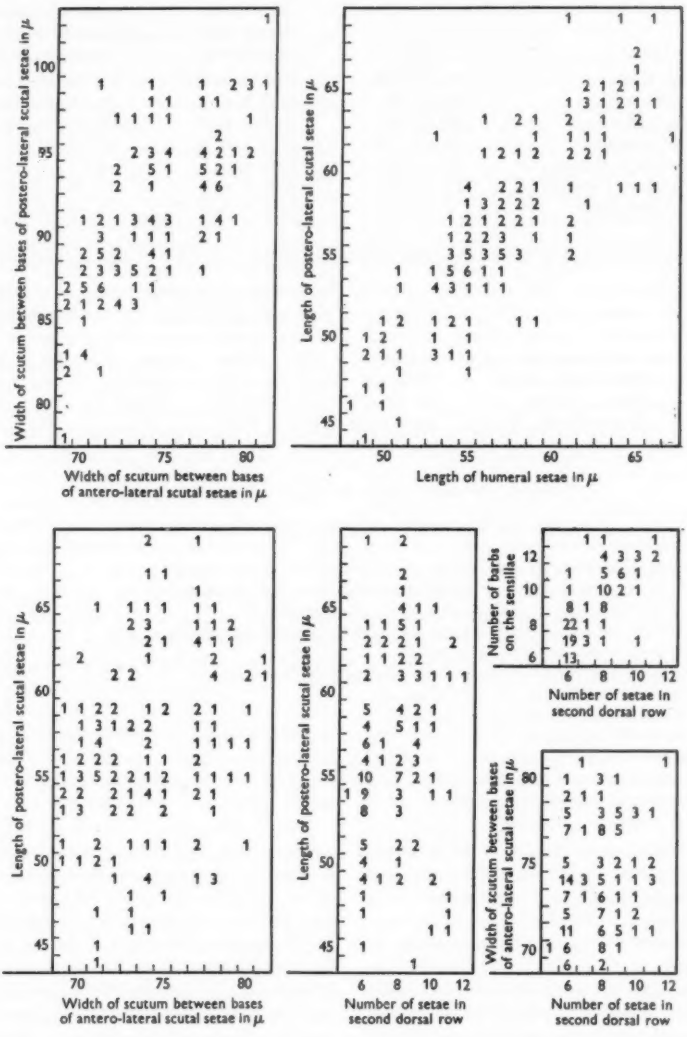
A, indicates that the sample contained more than 80 % type *a* larvae.
B, indicates that the sample contained more than 80 % type *b* larvae.
C, indicates that the sample contained more than 80 % type *c* larvae.
AB, *AC*, *BC*, indicates that the samples were mixed.
X, indicates the presence of larvae with duplicated postero-lateral scutal setae.
Y, indicates the presence of larvae with duplicated humeral setae.
Z, indicates the presence of larvae with duplicated antero-lateral scutal setae.
Percentages based on the characters shown by the first ten larvae to be examined in each sample. Larvae of type *X*, *Y* and *Z* shown even if the total number of larvae in the sample was less than ten.

setae may arise on one side of the animal but not on the other—a state of affairs which is even more pronounced in the case of duplicated scutal setae.

It therefore seems probable that variations in setal number, both on and behind the scutum, are governed by local conditions prevailing during the course of larval development and that the similarity

populations are differentiated on scutal width, no correlation can be obtained between this measurement and the other two. Moreover, samples with different scutal widths and postero-lateral scutal setal lengths may occur even in the same locality, as on the Fair Mile, near Streatley (compare samples taken in July with those taken in August and

Table 3. *Some correlations between scutal widths, setal lengths and setal numbers*


Width of scutum between bases of postero-lateral scutal setae in μ
Width of scutum between bases of antero-lateral scutal setae in μ
Length of postero-lateral scutal setae in μ
Length of humeral setae in μ
Length of postero-lateral scutal setae in μ
Width of scutum between bases of antero-lateral scutal setae in μ
Length of postero-lateral scutal setae in μ
Number of setae in second dorsal row
Number of barbs on the sensillae
Number of setae in second dorsal row
Width of scutum between bases of antero-lateral scutal setae in μ
Number of setae in second dorsal row

Measurements were taken to the nearest division of the eyepiece micrometer. At the magnification employed, seven micrometer scale divisions equalled eight μ's. The measurements plotted in Table 3 are given to the nearest μ. For this reason the table contains a space at every eighth μ in which no measurements are recorded.

Table 4. *The relation between the number of hairs in the second and third dorsal rows of the larvae and the length of the hairs on the posterior portion of the nymph*

Locality	Date	No. of hairs in second dorsal row of 5 larvae	Length in μ of the posterior hairs in 5 nymphs bred from same sample
Barley, Herts	14. viii. 46	6, 6, 6, 6, 6	12, 14, 13, 14, 13
Lydham, Salop	14. ix. 46	6, 6, 7, 6, 6	12, 14, 15, 13, 14
Tealfy, Lincs	23. ix. 46	6, 6, 6, 6, 6	14, 15, 14, 14, 15
Vaynol, Caerns	19. viii. 46	6, 6, 8, 8, 8	14, 15, 15, 6, 7
Muir of Ord, Ross	3. ix. 46	8, 8, 8, 8, 8	4, 7, 6, 5, 7
Puddington, Cheshire	13. ix. 46	8, 8, 8, 8, 8	7, 4, 5, 6, 6
Cowbridge, Glam	7. ix. 46	6, 6, 6, 6, 6	6, 7, 9, 8, 8

Table 5. *Ratio of length to depth in the tibia and tarsus of the first pair of legs in nymphs and adults*

			Length of tibia / Width of tibia			Length of tarsus / Width of tarsus		
Stage	Locality	Date	No.	Mean	S.D.	No.	Mean	S.D.
Nymphs type *a*	Muir of Ord, Ross	3. ix. 46	20	1·23	±0·15	20	2·25	±0·26
Nymphs type *b*	Barley, Herts	14. viii. 46	20	1·12	±0·21	20	2·13	±0·19
Adults	Clipperdown, Herts	June, July, 1946	20	1·21	±0·25	20	2·25	±0·16

Table 6. *Measurements of the larval type specimen*

Warren Farm, near Streatley, Berkshire, 2. x. 46

	μ
Width of scutum between bases of antero-lateral scutal setae	70
Width of scutum between bases of postero-lateral scutal setae	87
Depth of scutum from median seta to hind margin	42
Distance between antero-lateral and postero-lateral scutal setae	28
Distance between bases of sensillae	31
Distance from sensillae base to greatest depth of scutum	20
Length of antero-median scutal setae	40
Length of postero-lateral scutal setae	58
Length of humeral setae	57
Length of dorsal setae	50
Dorsal setal formula = 2, 6, 6, 6, 4, 4, 4	
Number of ventral setae = 32	

October, Fig. 7). Thus each combination of scutal width and setal length is due either to the existence of a genetically distinct local race, or, as in the case of setal number, variation in these characters is determined by the nature of the environment during larval development.

The latter is perhaps the more likely since phenotypic aberrations, namely ciliated galeal setae, duplicated postero-lateral scutal setae and duplicated humeral setae tend to be associated with characteristic scutal and setal measurements even though the larvae concerned may come from widely separated localities.

As to the factors influencing these characters, the presence of ciliated galeal setae or of particular scutal or setal dimensions cannot readily be correlated with any obvious climatic or seasonal influence, and the lack of correlation between variation in scutal width, variation in setal length and variation in setal number suggests that either different factors influence different types of variation or that these characters are determined at different stages in the development of the larvae. In the case of variations in setal number, their distribution in space and time is indicated in Table 2. Each sample examined was first referred to the 100 km. square of the national grid in which it happened to lie. Where known, the altitude at which the larvae were taken was indicated. The samples were further divided into those taken during the 25 days from 8 August to 1 September, and into those taken 50 days later during the 25 days from 22 October to 15 November. Considering the earlier period first, all the larvae from Scotland had numerous dorsal setae (type *a*). Further south, in Yorkshire, samples consisting wholly or partly of individuals with only six or seven setae in the second

and third dorsal rows began to appear (type *b*). Still further south, in the zone which stretches from Lincolnshire to North Wales, harvest mites with numerous setae were only found west of Derby. South of this, such larvae were only found in South Wales and became more numerous with increasing altitude, until they dominated a sample from 1500 ft. Aberrant forms were only found from 400 ft. upwards. Only type *b* and type *c* larvae were found in southern and western England.

By the end of October the eastern counties still showed samples composed mainly of type *b* larvae with few dorsal setae. Type *a* larvae, with numerous dorsal setae, became progressively more numerous towards the north and west of the country, appearing in areas from which they had been absent earlier in the year. This trend was associated with a fivefold increase in the proportion of aberrant types (namely duplicated postero-lateral and antero-lateral scutal setae and duplicated humeral setae).

In this way the number of setae tend to increase with altitude, lateness of the season, and the distances the sample lies towards the west and north. Duplication of humeral and scutal setae seems to appear in an accentuation of those conditions associated with increase in the number of post-humeral dorsal setae. This suggests that the factors governing the number of setae may be rainfall and temperature.

To sum up, in my view the differences observed between wild populations of larval harvest mites taken at different times and at different places are not due to the existence of genetically distinct races or species but are determined by the nature of the environment during larval development.

Since I have been unable to breed the British harvest mite, the evidence discussed above is solely circumstantial; until larvae of known ancestry can be reared under controlled conditions the hypothesis that there is but one species of harvest mite in Great Britain can neither be disproved nor confirmed.

NYMPHAL AND ADULT VARIATION

The samples of larvae from which nymphs were obtained were divisible into those consisting solely of larvae with numerous dorsal setae (normal type *a*), those consisting solely of individuals with few dorsal setae (normal type *b*) and into those in which both types of larvae were present. Nymphs reared from such samples showed no variations which could be correlated with differences in larval chaetotaxy. They agree with the description given by André (1930), save that the hair at the base of the palpal claw is simple not ciliated, the hair at the base of the tarsus is ciliated and not simple, and there is a stout spine in the centre of the inner side of the tarsus (Fig. 20).

Nymphs have been associated with their cast larval skins and an adult with its cast nymphal skin. Adults differ from nymphs in the greater ciliation of the hypostome, the presence of three spines not two at the base of the palpal claw and in having three pairs of genital disks instead of two (Figs. 11, 13, 14, 18, 21 and 22).

Adults were taken in the soil of rabbit warrens at Aldbury, Hertfordshire, on the Fair Mile, near Streatley, Berkshire, and in Vaynol Park, Caernarvonshire. On the Fair Mile they were associated with larvae differing in setal count. No variation corresponding to differences in larval chaetotaxy was observed in the adults. The genitalia, however, appear to fall into two types. In one the opening is a simple slit and is associated with the presence of unlaid eggs. In the other, the opening between the three pairs of genital suckers is occupied by what appeared to be a flask-shaped intromittent organ. I assume the first type to be the female genitalia and the second type the male genitalia (Figs. 22 and 23). Apart from this sexual difference, one individual from Vaynol Park had an aberrant crista, but otherwise resembled a normal adult.

In *Trombicula* the following characters are used to separate species of adults and nymphs: the chaetotaxy of the tibia of the palp, the shape of the crista, the nature of the genitalia, the ciliation of the epistome, the structure of the dorsal setae (Figs. 8–23) the length of the dorsal setae (Table 4) and the ratio of the length to the depth in the tarsus and metatarsus of the front pair of legs (Table 5). These characters are given in the accompanying tables and figures. In the nymphs there appeared a tendency for type *a* larvae to be associated with nymphs with short posterior hairs and for type *b* larvae to be associated with nymphs possessing long posterior hairs (Table 4). However, larvae taken in Glamorgan and in Radnor gave short-haired nymphs although the sample appeared to consist exclusively of type *b* larvae. This further suggests that the number of dorsal setae in the larvae is in part plastic.*

SPECIATION

As suggested above, the different types of British harvest mite larvae may be only phenotypic variations of a common genotype. Furthermore, though short-haired nymphs are normally associated with type *a* larvae and long-haired nymphs with type *b* larvae, short-haired nymphs have been bred

* The mean length of the posterior setae in twenty adults taken at Clipperdown Farm, Aldbury, Hertfordshire, on 10 June and 15 July, 1946 = 10·5 μ. S.D. of mean = $\pm$ 0·86 μ.

The mean length of the posterior setae in seven adults taken at Wood House Farm, Catterlen, near Penrith, Cumberland, on 2 April, 1948 = 9·7 μ. S.D. of mean = $\pm$ 1·52 μ.

from type *b* larvae. Similarly, adults collected in areas supporting several types of larvae cannot be separated into different races.

It seems highly probable, therefore, that there is but one species of harvest mite in Great Britain.

The first European species was described by Shaw in 1790 as *Acarus autumnalis*. Although no type specimen is known, there is no doubt that his description applies to the subject of this paper. As this name has priority over all other names for British harvest mites and since only one species of harvest mite is known to occur in Great Britain, I propose from my British material to select a larva as a lectotype. This lectotype is deposited in the British Museum of Natural History under the name *Trombicula autumnalis* (Shaw, 1790). The larva selected has simple galeal setae, solitary scutal and humeral setae and a dorsal setal count of 2, 6, 6, 6, 4, 4, 4. The type locality is the Fair Mile, Warren Farm, Streatley, Berkshire. For representative measurements see Table 6.

The taxonomic position of this species is difficult to determine. Circumstantial evidence derived from the examination of samples of larvae collected in the field, proved inconclusive. For example, although the larvae contained in five samples from four different localities could be separated on differences in setal ciliation, setal number and scutal and setal measurements into eight morphologically distinct types, on examination of further material these variations appeared phenotypic in origin and of no taxonomic importance. None the less, until larvae of known ancestry can be reared under controlled conditions, the systematic significance of the variations described above remains in doubt. Therefore I am unable to discuss the relationship of this species to those described by other authors.

SUMMARY

Five main types of harvest mite larvae have been found in Great Britain. These may be distinguished according to the following key:

(1) *Galeal setae ciliated*
Galeal setae not ciliated . . . . (2)

(2) *Scutal setae duplicated*
Scutal setae not duplicated . . . (3)

(3) *Humeral setae duplicated*
Humeral setae not duplicated . . . (4)

(4) Eight setae in both the second and the third rows of dorsal setae . Normal type *a*
Six setae in both the second and third rows of dorsal setae . Normal type *b*
Eight setae in the second dorsal row but only six in the third . Normal type *c*

Type *a* and type *b* may be further divided into groups based on differences in scutal width and setal length. Nevertheless, scutal width, setal length and setal number are not correlated with each other. Were larvae to be separated into two species on the hypothesis that one of these characters (e.g. differences in setal number) is genetically determined, then the range of variation with respect to the other two characters (namely scutal width and setal length) would be the same for each species. Forms with duplicated humeral setae do not differ otherwise from the normal type. The ciliation of the galeal setae and the duplication of the scutal setae is not consistent and larvae of mixed or intermediate type are known. It seems to me that these morphological differences may not be genetically determined but are solely phenotypic (and in the case of setal number may be influenced by rainfall and temperature). This, together with the lack of points of distinction among nymphs and adults, suggests that there is only one British species which was first described as *Acarus autumnalis* (Shaw, 1790). As no type is known to exist, a lectotype has been selected from British material and deposited in the British Museum.

The systematic position of this species is difficult to determine. Until larvae of known ancestry can be reared under controlled conditions the systematic significance of the variations described above must remain in doubt.

Nymphs and adults do not appear to differ greatly from those described by André (1930). The adult is recorded for the first time in England. The male genitalia are described.

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THE DISTRIBUTION AND BIOLOGY OF THE HARVEST MITE IN GREAT BRITAIN (TROMBICULIDAE, ACARINA)

By W. S. RICHARDS, M.A., D.Phil., F.R.E.S.

(With 3 Figures in the Text)

INTRODUCTION

In 1790 Shaw described the British harvest mite and gave it the specific name of *autumnalis*. To his description of the appearance, life history and biology of the larva little was added for over a hundred years, though the larva was described more accurately by Hirst (1915). He was also the first to rear recognizable nymphs of the British harvest mite (Hirst, 1926). This was repeated by André (1930) and Keay (1937). André obtained a small number of adults from the field. He also summarized and amplified previous knowledge of the biology and behaviour of the larvae, added numerous host records, both of mammals and birds, and described the peculiar lesion made by the larva in the tissues of its host—the 'histiosiphon' or 'autostyle' which had long been confused with the mouthparts.

Further investigations on the seasonal abundance of the harvest mite in Great Britain were made by Elton & Keay (1936), who studied the infestation on field mice in Bagley Wood, near Oxford. On the bank vole (*Clethrionomys glareolus* Schreiber) larvae were most abundant in early September. Their numbers then declined until they were entirely absent in June. They then increased to the September maximum. However, field voles (*Microtus agrestis* Bellamy) and long-tailed field mice (*Apodemus sylvaticus* L.) were only infested during the autumn. Further work by Keay (1937) showed that the harvest mite was more widely distributed throughout the British Isles than had been commonly supposed, and though most abundant in chalky districts, was recognized as a local pest as far north as the Island of Bute in the Clyde.

In my previous paper (Richards, 1949) I discussed the different types of harvest mite found in Great Britain. The larvae appeared to fall into two main groups: type *a* larvae with eight (or more) setae both in the second and in the third row of dorsal setae, and type *b* larvae with only six in each row. The former type appears to be associated with nymphs bearing setae about 6 μ in length on the posterior portion of the body, the latter develops into nymphs whose posterior hairs are about 12 μ. However, short-haired nymphs have been bred from samples of type *b* larvae sent from Glamorgan and Radnor. A third form, type *c* with eight setae in the second row of dorsal setae but with only six in the third, occurred in a few areas in the south and west of England. Besides these, specimens with ciliated galeal setae, duplicated humeral setae or duplicated scutal setae may preponderate in single samples of larvae. It is not known whether these variations are phenotypic or genotypic and they will therefore be referred to separately. A lectotype of *Trombicula autumnalis* has been deposited in the British Museum and I am of the opinion that all the forms described above will prove to be referable to this species.

METHODS OF SAMPLING

(i) *For the numbers of free, ungorged larvae seeking hosts*

Light traps can be used to collect all the free, ungorged larvae present on a single square yard of dry soil or dry, short grass. The form developed by my assistant, Mr K. L. Cockings (1948), on the Fair Mile near Streatley, Berkshire, consisted of a metal cover, a yard square, with a central 2 in. hole capped by a glass collecting tube which was placed over the area. A cone of damp filter-paper bridged the gap between the ground and the glass tube. Larvae trapped under the cover crawled towards the daylight shining through the central hole and were caught in the glass tube. This method is not suitable in long grass or over damp soil since condensation of water within the cover traps the larvae before they reach the glass collecting tube.

Bait animals can be exposed in situations unsuitable for light traps, for example in bracken, long grass, or in bushes. Guinea-pigs proved most suitable since they neither bite, burrow nor climb, and can be confined in light, bottomless, folding metal cages. My assistant Cockings discovered that provided the temperature was above 55° F., guinea-pigs and hamsters could collect all the free ungorged larvae present in a single square yard of ground during the course of a day. At lower grass temperatures light traps, under which the temperature was about 10–30° F. above that of the air outside, collected more mites than did the guinea-pigs. My own observations on the behaviour of guinea-pigs when confined in cages a yard square, suggested that

the infestation acquired might be due to active mites finding relatively quiescent guinea-pigs. Below 55° F. the mites were not active enough to ensure that they all found the guinea-pig during the course of a day's exposure.

Under the same conditions, different species of bait animals collected different numbers of harvest mites. Guinea-pigs were the most efficient, then golden hamsters (Cockings, 1948).

The infestation on bait animals, therefore, depends both on the activity of the mites and on the activity and nature of the host. Provided the temperature is above 55° F., a single guinea-pig will collect all the larvae present in a single square yard of ground during the course of a day.

Although the use of guinea-pigs and light traps gives an absolute estimate of the numbers of free ungorged larvae present in the areas sampled, besides their other limitations, these methods require bulky apparatus and hence only a few square yards can be sampled at a time. They are not, therefore, suitable methods for rapid preliminary surveys, nor can they be used to detect small numbers of larvae.

A rough index of the infestation within a limited area can be obtained by observing the numbers of larvae on the ears of wild rabbits. Since harvest mites take only 2 or 3 days to engorge, after which they leave the host, this infestation must have been acquired within a limited time. When larvae are scarce, they tend to congregate in a little pocket inside the rabbit's ear, and since the rabbit ranges over a larger area than can be conveniently sampled by light traps, or caged guinea-pigs, larvae can be detected though their numbers may be small.

Furthermore, large numbers of ears can be sent through the post and, perhaps most important of all, the sender need only cut off the rabbit ears close to the head, give details of time and place and post them as soon as possible. Thus samples of rabbit ears can be sent in by correspondents acting solely on written instructions. In this way information about the abundance and variation of the British harvest mite was obtained at a minimum of time and expense.

As with bait animals, the numbers of larvae observed would depend on the range of the rabbits and on the numbers and activity of the larvae. To try and reduce these sources of error I asked for the ears of wild rabbits shot in corn fields at harvest time. It was hoped that the abundant cover would allow the rabbits to wander freely at all times of the day and that their range, in the few days before harvest time, would be mainly confined to the field in which they were shot. The rabbits are usually shot in the evening, i.e. samples from different fields are taken at the same time of day. Harvesting usually takes place after a period of fine weather, so the activity of the larvae should be high. Moreover, the ripening of a given crop depends to a large extent on the local climate, some localities being 'more forward' than others, so the larvae occurring at harvest time may well be at the same period of their annual cycle of abundance in all areas.

The samples were obtained through individual farmers and through the pest officers of the county war agricultural committees. So far as I could ascertain, only the locality and the nature of the soil had any marked influence on harvest mite numbers. No connexion could be found between the nature of the crop harvested, the crop the previous year, or of the numbers of rabbits per acre. These results will be discussed later.

The infestations observed on field mice caught in live traps are similarly governed by such unknown factors as the range and activity of the larvae and the range and activity of their hosts. Moreover, as the larvae do not attack at night, the traps must be set out after nightfall and inspected at the same time of day, preferably in the early morning, if the infestations on different mice are to be compared. This method requires personal attention and is of use mainly in areas without rabbits. I have recorded all the different morphological types of larval harvest mites from field mice as well as from rabbits (save those with duplicated postero-lateral scutal setae). Mice caught in break-back traps only give information about the type of larvae present. Newly attached and partially engorged larvae may leave a dead host in the course of a few hours, so no estimate of numbers can be made.

Dragging a cloth over grass or flagging will collect free, ungorged larvae which can be removed from the cloth by a modified light trap. This method has not been tested quantitatively.

To sum up, under certain conditions, the numbers of larvae present in a square yard of ground may be accurately estimated by the use of guinea-pigs and light traps. The infestation on the ears of wild rabbits gives a rough index of the population present in a limited area and during a limited space of time. Samples of ears are simple to collect by untrained correspondents and can be sent through the post. They thus provided a simple and inexpensive means of gauging the variation and relative abundance of harvest mites in Great Britain at little expense. Live trapping field mice is only of use in the absence of rabbits. Dead trapping of field mice gives no information which cannot be obtained from rabbit ears. Flagging is a promising method requiring further investigation.

(ii) *On the host, for gorged larvae*

After leaving their hosts, gorged larvae are positively phototrophic and may be collected from their hosts by means of a light trap (Cockings, 1948).

(iii) *In the soil*

Adults are covered by a dense pile of plumed hairs which entrap air and are not readily wetted with water. So if dry, friable soil, for example chalk loam, be thrown into water, any adults present float to the top. Nymphs appear to be wetted more readily and their numbers cannot be accurately estimated by flotation in water. This method is not suitable for wind-blown sand since the dense froth formed when such soils are thrown into water tends to obscure the mites. Clay soils break up slowly and may retain adults until they become waterlogged and so fail to rise.

Tests were made on the Salt-Hollick soil sampling machine. This first removes organic matter from the soil by floating it off in magnesium sulphate solution with a sp.gr. of 1·18, and then separates the arthropods from the vegetation by xylene, which wets arthropod cuticle. Ninety per cent of the Erythraeid mite *Belaustium murorum*, a species comparable in size and physical properties with adult harvest mites, were recovered from the soil samples into which they were previously introduced. This method will also extract nymphophanes and telophanes (metamorphosing larvae and nymphs) as well as nymphs, and gorged and ungorged larvae. The numbers of these smaller stages cannot be accurately estimated since they become obscured by the froth formed on the interface between the xylene and the magnesium sulphate solution. This froth is stabilized by small soil particles. I know no means of breaking it up.

Other methods of flotation and sieving cannot be applied as adults are too fragile and larvae too small.

Methods relying on heat and desiccation to drive arthropods out of the soil (e.g. Trägårdh's method) are not suitable. Adults lose water too quickly for such methods to be of use, and larvae can be extracted far more readily by light traps.

GEOGRAPHICAL AND SEASONAL DISTRIBUTION

Harvest mites are well known in the south of England. In other parts of Great Britain they are recognized as a pest but not always under that name. In the north, they are called bracken bugs or berry bugs; in Ireland, orange tawneys; but more often the irritation they cause is attributed to midges, gnats, or similar small black insects.

During 1946, 374 samples of rabbit ears were sent to me from different parts of the British Isles (the data are deposited in the British Museum). Of these, fifty-nine had no harvest mites. The number of samples received from each county is plotted on Fig. 1. The upper figure gives the number of samples with harvest mites, the lower the number without. The location of the samples and the degree of infestation is given in Fig. 2.

Harvest mites occurred in almost all the areas from which I received samples. They were very abundant on the Chilterns, in the Isle of Wight, mid-Wales, Cheshire, the Lake District, Fife and on certain Scottish Islands. They were very scarce in the Yorkshire Dales, in south Sussex, round Winchester and near the Wash. Negative findings from other areas are doubtful, either because the sample was sent in late in the year, or because the rabbit ears sent were not cut off close to the head.

So far as can be seen, throughout Great Britain, seasonal fluctuations in numbers follows the findings of Elton & Keay (1936). The main brood of larvae appears at the end of June. By the beginning of September their numbers are at a maximum. Very few remain active after the first frosts, though there may be a temporary rise in numbers in May. Adults containing eggs have been found both in spring and during late summer and autumn. It seems probable, therefore, that the spring larvae represent this year's hatching and are not stragglers from the brood of the previous year.

During the main season, from July to November, type *b* larvae appear first. By the end of the season they may be almost entirely replaced by type *a* larvae. In some areas in Scotland and the north of England type *a* larvae are present throughout the year, while towards the south and east type *b* may persist throughout the season. Type *c* appears sporadically throughout the season but only in the south and west of England. Aberrations involving duplication of humeral or scutal setae tend to appear in conditions favouring normal type *a* larvae—viz. increase in altitude, the distance the sample lies towards the west and north, and lateness of the season. They may occur singly or form most of the sample and have been recorded from widely separated localities. Larvae with ciliated galeal setae have not been found north of Oxford. They occur throughout the year. Larvae and nymphs from the Scotch Isles of Bute, Eigg, Coll and Colonsay do not seem to differ from mainland forms (Richards, 1949).

The two types of nymphs appear to differ slightly in distribution from the two main types of larvae. In South Wales and Radnor, short-haired nymphs have been bred from type *b* larvae. In general, they appear to be most numerous in autumn and winter. So far as I can judge from samples collected during June, July, December and March, the number of adults present at Clipperdown, near Aldbury, Hertfordshire, remained constant throughout the year (Table 1). This was confirmed by partial observations made on the Fair Mile, near Streatley, Berkshire, and at Wood House Farm, Catterlen, near Penrith, Cumberland.

Eggs were found in females during the spring,

Fig. 1. The number of samples of rabbit ears received from each county during 1946. Upper figures indicate number of samples with harvest mites; lower figures, the number of samples without harvest mites.

Table 1. *The numbers of adults per square yard found at different depths and at different dates in a rabbit warren on Clipperdown Farm, Aldbury, Tring, Hertfordshire. Samples taken down to chalk bedrock*

Date ...	10. vi. 46	15. vii. 46		6. xii. 47	9. iii. 48
Depth in in.	Adults/ sq.yd.	Adults/ sq.yd.	Depth in in.	Adults/ sq.yd.	Adults/ sq.yd.
0– 4	5	5	0– 6	0	0
5– 8	11	36	7–12	8	4
9–12	32	48	13–18	46	4
13–16	11	16	19–24	20	52
17–20	8	16	25–30	—	12
20–24	16	12	31–36	—	4
Total	83	133		74	76

summer and autumn but were absent in winter. Only a single egg surrounded by a shell was seen at a time. There was no sign of any organized embryo.

Fig. 2. The location of the samples of rabbit ears received during 1946 with the degree of infestation in each area.

LOCAL FACTORS INFLUENCING NUMBERS OF LARVAE

The numbers of harvest mites present in a given area may vary greatly from year to year. For example, in 1947 a garden in Bangor, North Wales, had very few larvae compared with previous years (direct observation by Prof. F. W. Rogers Brambell). In contrast, on the Fair Mile, near Streatley, Berkshire, larvae were as numerous in August 1947 as they were in August 1946 (about 100 to the square yard). Larvae have been found at least 20 yards from a rabbit warren. In contrast, when a 40-yard transect was taken through a warren on Starveall Farm, Streatley, forty-six adults were found within the

18 yards of the transect which passed through the warren compared with only six in the 22 yards outside the warren. No obvious correlation was observed between the number of adults present and the presence of rabbit holes, nettles, or other features within the warren. The transect consisted of a single line of samples $\frac{1}{16}$th yard square spaced a yard apart, which were examined for adults by flotation in water. The confines of the warren were clearly marked out by the rank vegetation and the greater depth of soil.

Although larvae are very numerous round old-established rabbit warrens on dry chalk downs as on the Chilterns, they are equally abundant in more northern areas and on other types of soil, for example, on soils derived from Ordovician formations in mid-Wales (Newquay, Cardigan and Meifod, Montgomery) and in the Lake District (Braithwaite near Keswick), on earthy clay glacial soils overlying Carboniferous Limestone (Catterlen, near Penrith, Cumberland), on Boulder Clay overlying Triassic rocks in the Wirral (Puddington, Cheshire), and in Scotland, on soils overlying various igneous formations in Fife (parish of Dunbog) and on the Isle of Eigg; on Precambrian rocks on the Isle of Coll, and on Metamorphic rocks on the Island of Colonsay. They are not restricted to a given type of vegetation. Adults have been found both on open downland and in beechwoods supporting no undergrowth whatever. Since the majority of my conclusions are drawn from a study of the infestations found on rabbit ears, the texture of the soil where the larvae appeared to be most numerous is light and well drained, for this type of ground is preferred by rabbits. However, rabbits shot in corn-fields on light soils appeared to be more heavily infested than were rabbits from the same locality but from fields on heavy soils. Possibly a study of some area containing widely diversified soils, for example the Vale of the White Horse, Berkshire, might throw further light on this problem.

So far as the different forms of harvest mites are concerned, type *b* larvae were very abundant only on the chalky Chilterns, though they were fairly numerous on non-chalky soils and even on clays. Type *a* larvae were responsible for the very heavy infestations recorded away from the chalk. Heavy infestations of type *a* larvae were also found on the chalk of the Berkshire downs though later in the year than the main type *b* infestation. In my previous paper (Richards, 1949) I showed that type *a* larvae tended to replace type *b* larvae with lateness of season, distance towards the north and west, and increase of altitude (Fig. 3). I suggested that these two forms might be phenotypically determined, type *a* occurring under colder and more humid conditions than does type *b*. I am therefore unable to decide whether warm, dry, chalky soils favour type *b* more than type *a* or whether such larvae show the characters of type *b* when they develop in warm, dry, chalky soils, and type *a* when they develop in a rather colder, moister environment. If this last be true, then neither climate, nor the geological origin of the soil has any direct influence on the harvest mite infestations within the British Isles.

BEHAVIOUR

In the field, the activity of the larvae appears governed by the weather. They are most active when it is dry and sunny and least active when the day is cold and wet. When the temperature was below 55° F., guinea-pigs and hamsters failed to collect as many larvae as did light traps (the temperature beneath which was usually about 10–30° F. higher than the grass temperature) (Cockings, 1948).

The preattachment larvae tend to congregate in groups on the top of certain small clods of earth while other similar clods are untenanted. After 3 weeks without rain they were found to be most numerous under bushes where the ground was less dry and they appeared to prefer lumps bearing small patches of moss. They are very strongly positively phototropic and if a light be directed upwards through the floor of a glass tube in which they are confined, they will walk rapidly down the tube towards it. As mentioned above, I believe they find suitable hosts partly through their own efforts, though whether this takes the form of a directed response or whether it is solely by random movements I do not know. This might be determined by comparing the numbers of larvae collected round caged guinea-pigs surrounded by paper soaked in di-butyl phthalate (which acts as a sticky trap) with those collected on similar strips of paper without the guinea-pigs present.

Even in warm dry weather larvae are not active at night. In experiments at the end of August 1946, on the Fair Mile, near Streatley, Berkshire, guinea-pigs set out at 5 p.m. in the afternoon were mainly infested with gorged larvae when examined at 10 a.m. on the following day. Similar guinea-pigs set out after nightfall (9 p.m.) had only engorged larvae on them when visited at 10 a.m. next morning. It therefore seems probable that although the second lot of guinea-pigs were exposed all night, they were only attacked after dawn.

So far as I can tell, the larvae appear to infest any warm-blooded vertebrates with which they may come in contact. I have personally seen them on field vole (*Microtus agrestis* Bellamy), long-tailed field mouse (*Apodemus sylvaticus* L.), house mouse (*Mus musculus* L.), grey squirrel (*Sciurus carolinensis*, Gmelin), rabbit (*Oryctolagus cuniculus* (L.)), hare (*Lepus europaeus* Pall.), stoat (*Mustela erminea* L.), hedgehog (*Erinaceus europaeus* L.), and have seen specimens from brown rat (*Rattus norvegicus*

Fig. 3. The influence of geographical position and time of year on the ciliation of harvest mites: *A* for ninety-six samples received from 8 August to 1 September, 1946. *B* for thirty-two samples received from 22 October to 15 November 1946. The maps are marked out in the 100 km. squares of the national grid. Areas from which no samples were obtained are left blank. Black areas = normal type *a* present. Stippled areas = normal type *b* present. Areas with horizontal black and white bands = normal type *c* present. Areas with blank circles = aberrant forms present, i.e. double antero-lateral scutal setae; double postero-lateral scutal setae; double humeral setae.

Erxleben), common shrew (*Sorex araneus* L.) and house sparrow (*Passer domesticus* L.). However, there are preferences. When exposed to infestation, guinea-pigs and golden hamsters collected the most larvae, followed by rabbits and white mice (Cockings, 1948).

On the host the larvae tend to aggregate in little clusters, sometimes about the size of a sixpence. If the numbers are few, the ears appear to be attacked first. When the infestation is heavy other parts of the body are attacked, particularly the anus and genitals. (In the house mouse the genitals appeared most favoured.) Even in the same species the same parts of the body are not always attacked. In young male guinea-pigs exposed on the Fair Mile, Streatley, Berkshire, distribution of harvest mites was by no means uniform. Although the perineum and coccyx were most favoured, individuals occurred with most of the infestations on the ears, or even on the teats. Scattered patches were sometimes found on other parts of the body (Table 2). It seems, therefore, that the mere act of attachment by one larva makes that area more attractive to other larvae. Thus some areas become covered with tightly packed larvae, but others, apparently just as suitable, remain unattacked.

Table 2. *Number and location of harvest mites on ten individual guinea-pigs exposed on the Fair Mile, near Streatley, Berkshire*

Date	Location of mites					
	Left ear	Right ear	Perineum	Coccyx	Teats	Other places
28. viii. 47	53	20	10	16	0	0
	9	24	6	4	0	0
	4	2	15	0	0	0
	9	12	13	42	0	0
	48	32	34	37	0	35 on hind foot
21. viii. 48	5	0	0	0	0	0
	0	2	1	0	0	0
	2	0	13	0	0	0
	0	0	0	23	0	0
	18	7	1	0	4	0

The larvae take 1–3 days to engorge. They then leave their host. For the first 2 or 3 days the gorged larvae are strongly phototropic. They then descend into the soil and remain quiescent while the nymphs develop within the larval skin. The nymphs have been seen to suck the contents of pupating larvae (nymphophanes). Both this stage and the adult spend much time with the two long front legs raised at an angle of 30° from the horizontal. Warm moist weather appears to bring the adults near the surface of the soil, heavy rain, drought and frost drive them down (Cockings, 1948). They may descend at least 3 ft. and appear to congregate where the soil is palpably moist. In winter the majority of adults have been found in a stratum of soil between 18 in. and 2 ft. deep. So far as I can tell, they control their water balance by migrating to regions of suitable aqueous tension. They dry up in dry atmospheres and appear to absorb water and swell up under very humid conditions. More precise data might be obtained by observing the pF which they prefer. The pF is a measure of the fugacity of water vapour in the soil and is analogous to osmotic pressure with respect to aquatic animals and to relative humidity with respect to aerial animals. They have been kept in damp peat at a temperature of 30° C. for 6 weeks.

SUMMARY

Previous work is discussed and described. The numbers of larvae present in a square yard of soil can be estimated by the use of caged guinea-pigs or by light traps. The infestation observed on the ears of wild rabbits gives a rough index of the population present in a given area.

Using a modified light trap, gorged larvae can be collected from their hosts.

Adults can be extracted from light, friable soils by flotation in water and from any type of soil by the Salt-Hollick soil-sampling machine.

The harvest mite occurs throughout the British Isles. It seems possible that climate and the geological origin of the soil do not affect harvest mite abundance. They are, however, less numerous on heavy clays.

Larvae are most abundant in late summer and autumn. Adults remain constant in numbers throughout the year.

The larvae appear to infest most warm-blooded vertebrates. They do not attack at night. Nymphs have been observed sucking the contents of nymphophanes.

Adults have been found at a depth of 3 ft. They

to 1 September, 1946. *B* for thirty-two samples received from 22 October to 15 November 1946. The maps are marked out in the 100 km. squares of the national grid. Areas from which no samples were obtained are left blank. Black areas = normal type *a* present. Stippled areas = normal type *b* present. Areas with horizontal black and white bands = normal type *c* present. Areas with blank circles = aberrant forms present, i.e. double antero-lateral genital setae; double postero-lateral genital setae; double humeral setae.

appear to come nearer the surface in warm, moist weather, but are driven down by drought, heavy rain and ground frost.

In my previous paper (Richards, 1949) I made acknowledgement to all those people without whose generous assistance this work could not have been completed, and to whom I now express my hearty thanks.

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OBSERVATIONS ON THE VERTICAL MIGRATIONS OF THE THIRD-STAGE LARVA OF *HAEMONCHUS CONTORTUS* (RUD.) ON EXPERIMENTAL PLOTS OF *LOLIUM PERENNE* S24, IN RELATION TO METEOROLOGICAL AND MICROMETEOROLOGICAL FACTORS

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(With 3 Figures in the Text)

During recent years an increasing amount of work has been done on the biology of the third-stage larvae of some of the species of nematodes which infest grazing animals. Among the writers who have contributed to these studies are Morgan (1929), Schwartz, Imes & Wright (1930), Monnig (1930), Lucker (1936), Taylor (1938*a*, *b*, 1939), Rogers (1940), Buckley (1940), Kauzal (1941), Shorb (1942) and others. Most of the investigations have been made on sheep and horse strongyles, and there is much information of a general nature concerning the tropisms of these larvae and their behaviour in faeces and soil. One of the most comprehensive papers dealing with the effect of environmental conditions on the movements of infective larvae on grass is that by Rogers (1940), who deals with factors influencing the accessibility of third-stage trichostrongyle larvae to grazing animals. Further work is, however, necessary to determine exactly the behaviour of the larvae under different conditions of temperature, light and humidity, in their immediate environment. This environment, constituted by the surface layer of the soil and the covering of vegetation, is obviously a restricted one which will have a climate of its own differing from that of the outside environment. The study of such a restricted climate is described as microclimatology, although micrometeorology is a better term, the former being essentially a fair-weather study. Any observations made on the movements of free-living nematode larvae should therefore be correlated with the micrometeorological factors of the immediate environment as well as with the meteorological factors of the outside environment. Variations in temperature and humidity at ground level can be astonishing in their magnitude. The temperature of the surface of the soil varies considerably; in warm weather it gains heat during the day and loses it by radiation at night, the reverse being true in winter. The variations are less marked when there is a close cover of vegetation; even so, vegetation makes its own climate where the temperature and humidity will differ from that at, say, 6 ft. above the ground. In the present paper, therefore, all movements of the larvae have been correlated with micrometeorological factors.

One species, namely, *Haemonchus contortus* (Rud.), has been chosen for a detailed examination of the behaviour of the infective larvae in different circumstances. The whole scheme of study has been arranged in three sections: (1) outdoor observations on specially prepared experimental plots, (2) laboratory experiments, and (3) field experiments. Section (1) has been completed, and the results are embodied in the following pages.

OUTDOOR OBSERVATIONS

Observations were continued over a period of one year, from December 1946 to November 1947. They were made on specially prepared grass plots on one day in each month of the year. Except where otherwise stated a fine day was chosen for each occasion. Observations were made at 2-hourly intervals throughout the 24 hr. period, commencing at 2 a.m. G.M.T. and continuing until midnight. Each observation consisted primarily of the recovery and counting of nematode larvae from the grass and surface of the soil. At the same time meteorological and micrometeorological observations were made, namely, the temperature of the air at 6 ft., the temperature at ground level, the temperature of the soil 2 in. below the surface, and the percentage relative humidity at 6 ft. and at ground level. The intensity of the light was also measured. The collected data were then examined to ascertain whether there was any correlation between the vertical migrations of the larvae and the meteorological and micrometeorological factors.

METHOD

For each series of observations twelve grass plots were necessary, one for each 2 hr. period during the day. Six unglazed earthenware pots measuring 10 in. square were used, each being divided later into two. The pots were filled with steam-sterilized soil; the

top inch of soil was then removed from each pot into separate containers, and fertilizers at the rate of 2·5 g. superphosphate and 1·0 g. ammonium sulphate added to each and thoroughly mixed with the soil. Half of this soil was then replaced in each respective pot and spread over the surface forming a layer half an inch deep. Grass seed was then sown evenly over this at the rate of 6·0 g. per pot. The other half of the soil was then spread over the grass seeds and pressed down firmly. Each of the six plots was then divided in half by a sheet of glass sunk into the soil and projecting a short distance above the surface, giving twelve plots measuring 10 × 5 in. each with a surface area of 50 sq.in. An additional plot was made on each occasion for use with the earth thermometer.

Various species of grasses were tried before the experiments commenced. These were *Phleum pratense* S 48/BF/45 (Timothy), *Dactylis glomerata* S 143/FC/45 (cocksfoot), *Lolium italicum* Bb 440 (National Italian ryegrass), and *L. perenne* S 23 and S 24 (perennial rye grass). It was found that *L. perenne* S 24 was the most suitable both for rate of growth and diameter of leaf; this species was therefore used throughout.

The preparation of the plots was carried out in the laboratory, where they remained until they were required out of doors. Each received an exactly equal amount of water, 40 ml./day, which was sprayed over the surface with a fine syringe. The time taken for the grass to grow varied slightly at different times of the year, the time of sowing was therefore adjusted accordingly. An adequate growth could be obtained in a maximum period of 3 weeks at any time. The plots were used before the grass tillered, so that the 'pasture' consisted in all cases of individual simple blades. Before the plots were used the grass in each was cut to a uniform height of 3 cm.

On the afternoon of the day previous to that on which the observations were to be made, the plots were conveyed out of doors in order that they might become acclimatized to the conditions of temperature and humidity existing there. The same position was used in each successive month.

At midnight each of the twelve plots was infected with 1200 third-stage larvae of *Haemonchus contortus*, the number being the same throughout the year for each plot. The larvae, suspended in 5 ml. of water, were distributed evenly over the surface of the soil at the bases of the grass blades, a fine glass pipette being used for the purpose. None was placed actually on the grass blades. A thermometer and a wet- and dry-bulb hygrometer were suspended 6 ft. above the ground near the plots, and another wet- and dry-bulb hygrometer placed level with the top of the grass in plot 1. A terrestrial radiation thermometer was supported horizontally just above the grass in the same plot, and an earth thermometer in a spare plot alongside with the bulb 2 in. below the surface. After a lapse of 2 hr., at 2 a.m., the readings of the various instruments were recorded and the hygrometer and terrestrial radiation thermometer moved on to plot 2. The first plot was taken up and the grass cut in two lengths, the top 2 cm. and the bottom 1 cm., with a pair of scissors. The top 5 mm. of soil was then removed with a flat-bottomed 'spoon' 3 in. in diameter. This procedure was continued throughout the 24 hr. period, a fresh plot being taken at the end of each 2 hr. The terrestrial radiation thermometer was not left in position during bright sunshine; in such a circumstance it was placed in position long enough to record the temperature. During the hours of daylight the intensity of the light was measured with a G.E.C. lightmeter, which recorded intensities of from 0 to 1000 f.c. At some times during the year, mainly during the winter months, the light intensity could be measured throughout the day, but on bright summer days it could be measured only during the morning and evening, as the intensity exceeded 1000 f.c. during the middle of the day. Dim light, however, seems to be of greater significance in stimulating upward migrations than bright light, as will be seen from the following results.

Extraction of larvae from the samples was begun immediately, the Baermann technique being used. Sieves of 60 mm. mesh were placed in the funnels which were filled with water at 30° C., as the larvae seem more active at this temperature. The samples of grass and soil were left in the funnels for 36 hr.; 5 ml. of water were then drawn off from the bottom of each funnel into a graduated tube and all the contained larvae counted.

As pointed out by Dinaberg (1942) this method of extraction is not entirely satisfactory, as the percentage recovery is not very high. In the present case, the method of procedure and the initial number of larvae being constant throughout, the results are probably comparable.

RESULTS

The results of each monthly observation are shown in the tables which follow. All the data are included in the tables: the light intensity; the variations between the temperature and humidity at ground level and at 6 ft. above the ground; the temperature of the soil at a depth of 2 in.; the number of larvae recovered from the bottom 1 cm., the top 2 cm. of the grass blades, and the total number of larvae on grass; and the number of larvae in the top 5 mm. of soil.

Observation 1, 2 *December* 1946 (Table 1). Dull, cloudy day.

Temperature. The temperature at ground level varied by only 0·5° C. during the first 12 hr., the maximum of 8·5° C. occurring at 10 a.m. In the

Table 1. *2 December* 1946. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at 2-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 c.m of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	9·4	8·0	8·9	64	59	Nil	27	5	32	360
4 a.m.	9·4	8·0	8·9	64	59	Nil	35	15	50	181
6 a.m.	9·4	8·0	8·9	64	53	Nil	42	12	54	150
8 a.m.	8·9	8·0	8·9	63	77	17	129	10	139	13
10 a.m.	8·9	8·5	8·3	63	73	560	174	22	196	38
12 noon	8·9	8·0	8·3	56	73	920	58	16	74	25
2 p.m.	8·9	7·5	8·3	56	77	330	66	14	80	75
4 p.m.	8·3	7·5	7·8	58·9	73	50	95	25	120	75
6 p.m.	8·3	7·0	7·2	62·5	80	Nil	54	15	69	38
8 p.m.	7·8	6·5	7·2	100	96	Nil	59	10	69	50
10 p.m.	7·8	6·5	7·2	92	96	Nil	42	8	50	101
12 m.n.	7·8	7·5	7·0	69	92	Nil	11	6	17	169

second half of the day, the temperature fell gradually to 6·5° C. at 8 and 10 p.m., rising to 7·5° C. at midnight. The temperature of the air at 6 ft. was slightly higher than at ground level, as was also in the majority of cases the temperature of the soil.

Humidity. The humidity at ground level was on the whole higher than that at 6 ft.; it increased throughout the day.

Light intensity. This factor seemed the most variable. There was a long period of darkness. At 8 a.m. the light intensity measured 17 f.c.; it rose gradually to a maximum of 920 f.c. at noon and fell to 50 f.c. at 4 p.m.

Total number of larvae on grass. Only small numbers of larvae were recovered from the grass during the first 6 hr. During the next 4 hr. there was a gradual rise to a morning maximum of 196 at 10 a.m., followed by a fall during the middle of the day and a second rise to 120 at 4 p.m. The evening maximum was not as high as the morning. The majority of larvae were recovered from the bottom 1 cm. of grass. In no case did the number on the top 2 cm. exceed that on the bottom 1 cm., though the two maxima were apparent in both regions.

Larvae in top 5 mm. of soil. The number of larvae recovered during the first few hours was high; probably the majority were still on the surface of the soil after being placed there at midnight. During the day, the number recovered fell rapidly, rising again in the late evening. The fall during the day may be explained in part by the rise in numbers on the grass. It is also possible that some of the larvae penetrated the soil to a depth greater than 5 mm. and so were not recovered.

It is interesting to note the rise in numbers of larvae on the grass in the morning and evening. Considering the various factors which might cause this, light seems to be the most variable. The temperature was relatively even throughout and the humidity high. It seems possible, therefore, that provided these two latter conditions are favourable, light of a particular intensity or within a range of intensities may be the determining factor in the rise of larvae on the grass. This possibility will be reviewed later after the observations throughout the year have been considered.

Observation 2, 2 *January* 1947 (Table 2). Dull, cloudy day.

Temperature. The temperature at ground level was slightly higher than in December, rising from 7·5° C. to a maximum of 10° C. during the middle of the day, and falling again to 6·5° C. at midnight. The temperature at 6 ft. was, in each case, higher than that at ground level, and the temperature of the soil in the majority of cases lower.

Humidity. The humidity was higher at ground level than at 6 ft. and was higher than in the previous month.

Light intensity. The day was very cloudy, and so the maximum light intensity at midday was only 360 f.c. There was again a long period of darkness. The light intensity measured 15 f.c. at 8 a.m. and 110 f.c. at 4 p.m.

Total number of larvae on grass. At 8 a.m., when it began to get light, there was a marked increase in the number of larvae recovered from the grass, the maximum occurring again at 10 a.m. There was a fall during the middle of the day, and a second maximum, smaller than the first, at 4 p.m. The majority of the larvae were again recovered from the bottom 1 cm. of the grass.

Table 2. 11 *January* 1947. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at* 2*-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	9·4	7·5	7·8	71	96	Nil	60	3	63	211
4 a.m.	8·3	7·0	6·7	77	96	Nil	35	5	40	52
6 a.m.	8·6	7·0	6·7	70	92	Nil	23	7	30	62
8 a.m.	9·4	7·5	6·9	74	100	15	174	11	185	103
10 a.m.	9·4	9·0	7·5	74	74	140	185	15	200	73
12 noon	10·3	10·0	7·8	75	79	360	137	13	150	51
2 p.m.	10·3	10·0	8·9	79	82	320	92	10	102	88
4 p.m.	10·0	9·5	8·9	75	82	110	105	11	116	114
6 p.m.	8·3	7·5	8·9	77	88	Nil	29	6	35	98
8 p.m.	7·8	6·5	6·7	69	87	Nil	34	6	40	209
10 p.m.	7·2	6·0	6·7	76	91·5	Nil	28	4	32	201
12 m.n.	7·2	6·5	6·4	76	87	Nil	51	4	55	125

Table 3. 27 *February* 1947. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at* 2*-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	−1·1	−4·5	0·0	43	58	Nil	10	0	10	12
4 a.m.	−2·2	−5·0	−0·3	40	52	Nil	0	0	0	10
6 a.m.	−6·1	−5·5	−0·3	39	41	Nil	15	0	15	16
8 a.m.	−6·1	−5·0	−0·3	39	41	135	7	0	7	10
10 a.m.	−1·7	−2·5	−0·6	30	32	Above 1000	0	0	0	15
12 noon	1·1	−1·5	−0·6	31	33	Above 1000	6	0	6	6
2 p.m.	1·1	−1·0	−0·6	31	33	860	10	0	10	6
4 p.m.	1·1	−1·0	−0·6	31	27	120	8	0	8	9
6 p.m.	0·0	−2·0	−0·6	27	27	20	12	0	12	14
8 p.m.	0·0	−3·0	−0·6	27	27	Nil	10	0	10	17
10 p.m.	−0·6	−1·0	0·0	24	22	Nil	6	0	6	14
12 m.n.	−0·6	−1·0	0·0	24	22	Nil	9	0	9	11

Larvae in top 5 *mm. of soil.* The number of larvae recovered from the top 5 mm. of soil was again high at the beginning and end of the 24 hr. period and fell during the day.

Observation 3, 27 *February* 1947 (Table 3). Clear, very cold day.

Temperature. The temperature at ground level was below freezing-point for the whole of the 24 hr. It was lowest at 6 a.m., −5·5° C., and highest in the early afternoon and late at night, when it rose to −1·0° C. The temperature of the air at 6 ft. was, on the whole, above that at ground level, rising to 1·1° C. between noon and 4 p.m. The temperature of the soil showed less variation, ranging from 0 to −0·6° C., being lowest during the daytime.

Humidity. The humidity at ground level and at 6 ft. was low throughout and fairly similar in both cases. There was a steady fall in the humidity during the period of observation.

Light intensity. The light was more variable than on the two previous occasions. The duration of the light period was slightly longer and the increase in

intensity more rapid. At 8 a.m. it measured 135 f.c. and by 10 a.m. exceeded 1000 f.c. There was a gradual fall in the intensity during the afternoon to 20 f.c. at 6 p.m.

Total number of larvae on grass. The total number of larvae recovered from the grass during each 2 hr. period throughout the 24 hr. was extremely low, varying from 0 to 15. No appreciable rise or fall was evident, and no larvae succeeded in climbing beyond the first 1 cm.

Larvae in top 5 mm. of soil. Extremely few larvae were recovered from the soil, the numbers being somewhat smaller than those on the grass, no definite rise or fall was apparent.

The absence of any marked movements of the larvae in this case was probably associated with the very low temperature and low humidity. The light was at times favourable, but in the absence of other suitable conditions the larvae were unable to respond to it. It is well known that the infective larvae of *H. contortus*, like others of its kind, are not able to move except on a damp surface. The amount of moisture required is very small, but, with temperatures as low as those experienced here, any drops of moisture would be frozen rapidly, leaving the surface of the grass practically dry. The few which were recovered from the grass probably succeeded in getting on to the bases of the grass blades immediately after infection of the soil and remained there until extracted.

It seems, therefore, that low temperatures render the larvae less active, this inactivity being combined with an inability to climb a dry surface. Monnig (1930) found that infective larvae in a culture jar descended to the faeces when placed in a refrigerator at 0° C., and although still alive some days later they were unable to migrate upwards in spite of the return of favourable conditions. The failure to recover many larvae from the surface layer of the soil is, in view of this, difficult to explain. They may have been killed by the low temperatures, or they may have migrated to a greater depth, although this seems doubtful in view of the hardness and dryness of the soil.

It is evident from the observation that the presence of one suitable factor alone will not stimulate the larvae to climb. A combination of a certain range of temperature, humidity and light intensity seems to be necessary. Probably any one might be regarded as the determining factor if the others are favourable, although light, being as a general rule the most variable throughout any one day, would probably bring about a more marked reaction than either of the others.

Observation 4, 25 *March* 1947 (Table 4). Dull, cloudy day.

Temperature. The temperature at ground level varied over a fairly wide range, from 2·5° C. at the commencement of the period to a maximum of 12·2° C. at 2 p.m. The temperature at 6 ft., except for the middle of the day, was above that at ground level and less variable. The temperature of the soil can be seen from the table.

Humidity. The humidity was fairly high. It fell in the early evening when a slight breeze came up. There was a ground mist at 10 a.m., accounting for the humidity then reaching nearly to saturation point.

Light intensity. The light period was slightly longer than on the previous occasion, the first intensity being measured at 6 a.m. instead of at 8 a.m. The

Table 4. 25 *March* 1947. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at 2-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	4·4	2·5	3·3	73	75	Nil	50	5	55	179
4 a.m.	4·4	2·5	3·3	73	75	Nil	94	0	94	183
6 a.m.	3·3	2·8	3·3	76	85·5	15	105	50	155	88
8 a.m.	5·0	6·9	3·3	65·5	82	460	100	35	135	72
10 a.m.	11·1	11·4	8·6	66	96·5	Above 1000	104	20	124	121
12 noon	7·5	9·2	8·3	80·5	77	Above 1000	75	15	90	87
2 p.m.	10·0	12·2	8·6	59	52·5	Above 1000	45	5	50	88
4 p.m.	10·0	10·6	8·9	59	62·5	1000	55	15	70	93
6 p.m.	8·6	10·3	8·3	63	59	70	124	11	135	239
8 p.m.	7·8	10·6	7·8	69	56	Nil	85	16	101	156
10 p.m.	7·8	8·3	7·2	69	80·5	Nil	80	10	90	132
12 m.n.	7·8	8·3	7·2	69	80·5	Nil	54	6	60	247

increase in intensity was rapid and was maintained at over 1000 f.c. from 10 a.m. to nearly 4 p.m.

Total number of larvae on grass. At 6 a.m., with the appearance of light, the number of larvae recovered from the grass rose to a maximum of 155, followed by a fairly large number, 135, at 8 a.m. Thereafter there was a gradual fall followed by a rise to an evening maximum at 6 p.m. Again the majority of the larvae were recovered from the bottom 1 cm. of the grass. On the top 2 cm. of grass the morning maximum was apparent, but there was no definite indication of a rise in the evening.

Larvae in top 5 mm. of soil. The number of larvae recovered from the top 5 mm. of soil was again higher in the early morning and at night than during the daytime. This seems to be a fairly constant occurrence throughout.

As in December and January, the larvae climb up the grass blades in the morning and evening. The morning maximum in December and January occurred at 10 a.m., whereas here it occurred at 6 a.m. Similarly, the evening maximum which occurred in the earlier months at 4 p.m. occurred here at 6 p.m. The two peaks on the curve are separating from one another. The only apparent explanation for this seems to be the increase in length of the period of daylight, causing light of lower intensities to appear earlier in the morning and later in the evening. The temperature and humidity show no marked differences. It seems fairly evident therefore that other conditions being favourable the larvae respond to the stimulus of relatively dim light or to the first appearance of light after darkness. This latter may account for the fact that the morning maximum is higher than the evening, although this may possibly be bound up with humidity and temperature changes. The process of wider separation of the two maxima is carried still further later in the year, as will be seen in the observations which follow.

Observation 5, 29 *April* 1947 (Table 5). Dull, cloudy day.

Temperature. The temperature at ground level varied from 5·8° C. at 4 a.m. to 11·1° C. at 4 p.m. It was, in the main, below the temperature at 6 ft. except for the last few hours. The temperature of the soil was more uniform, varying between 7·2 and 10·6° C.

Humidity. The humidity was higher at ground level than at 6 ft., and rose to saturation point at 2 p.m. owing to a thick mist.

Light intensity. The duration of daylight was longer still, it being light from 4 a.m. until 8 p.m. As the day was very cloudy, the intensity did not exceed 1000 f.c., although it reached this level at 8 a.m. and at 4 p.m.

Total number of larvae on grass. The total number of larvae on the grass reached a morning maximum of 226 at 6 a.m. and an evening maximum of 172 at 8 p.m.; the evening maximum occurred 2 hr. later than in the previous month. Again the majority of the larvae were recovered from the lower portion of the grass blades, but the two maxima were evident in both regions.

Larvae in top 5 mm. of soil. The larvae in the top 5 mm. of soil were high in numbers at both ends of the period and lower during the day.

Table 5. 29 *April* 1947. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at 2-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	7·8	6·7	8·1	84	91	Nil	89	10	99	250
4 a.m.	7·2	5·8	7·2	76	88·5	25	108	12	120	265
6 a.m.	7·8	6·7	7·2	77	86	280	174	52	226	145
8 a.m.	9·4	10·8	7·8	74	80	1000	123	21	144	100
10 a.m.	10·3	9·4	9·4	63	72	660	79	20	99	97
12 noon	11·1	10·6	9·4	73	86	780	86	18	104	100
2 p.m.	10·8	10·0	10·0	96·5	100	640	116	19	135	122
4 p.m.	11·7	11·1	10·6	82·5	93	1000	118	20	138	120
6 p.m.	10·6	10·0	10·6	79·5	89	330	129	17	146	117
8 p.m.	8·9	10·6	9·4	72·5	69	15	142	30	172	150
10 p.m.	8·3	10·0	8·9	70	88·5	Nil	109	21	130	205
12 m.n.	8·3	9·4	8·9	80	74	Nil	87	7	94	200

Observation 6, 22 *May* 1947 (Table 6). The day was cloudy; thunder and sharp showers of rain continued intermittently from 9.30 p.m. until midnight.

Temperature. The temperature at ground level was, on the whole, higher than temperatures encountered previously, varying from 6° C. at 4 a.m. to 16·5° C. at 10 a.m. The temperature at 6 ft. was very similar and that of the soil higher.

Humidity. The humidity at ground level was fairly high. It rose during the last two observation periods as a result of precipitation.

Light intensity. The duration of daylight was the same as on the previous occasion, but the light reached a higher degree of intensity during the day, being above 1000 f.c. from 8 a.m. until noon.

Total number of larvae on grass. Neglecting, temporarily, the last two readings, namely, 10 p.m. and midnight, there were again two maxima, the first at 4 a.m. and the second at 8 p.m.

Larvae in top 5 mm. of soil. Neglecting again the last two readings, which will be referred to later, the number of larvae recovered from the soil was high at the beginning of the period and fell rapidly to a minimum at 8 p.m.

The most striking feature in this observation is the sudden and very marked rise in the number of larvae on the grass and in the surface layer of the soil from 10 p.m. until midnight. In both cases, a larger number of larvae was recovered than during any other time of the day in spite of the complete absence of light. This phenomenon has not occurred anywhere else during the whole series of observations. The only apparent circumstance which might have accounted for this sudden rise and which did not occur elsewhere throughout the year was the thunderstorm which began at 9.30 p.m. and continued intermittently for about 2 hr., there being several showers of fairly heavy rain to which the plots were left exposed. It seems, therefore, that there are two factors which might account for this rise of larvae, either the sudden increase in humidity or the electrical disturbances in the atmosphere. It is possible that the sudden wetting of the grass blades after a period of relative dryness might have stimulated the larvae to climb in spite of the absence of light. When, however, rain was continuous throughout the 24 hr., as was the case in July, this sudden rise in darkness was not apparent, the larvae behaving 'normally'. But rain following relatively dry conditions may have a different effect. The other possibility is that the electrical disturbances may in some way have produced this reaction, e.g. the increase in the oxygen in the atmosphere, although infective nematode larvae do not as a rule respond to increased amounts of oxygen, or the soil may in some way have been affected by the disturbances, causing a movement away from it on to the grass. The large numbers recovered seem to indicate that the larvae have emerged from the deeper layers of the soil.

Observation 7, 28 *June* 1947. (Table 7). Dull, cloudy day.

Temperature. The temperature at ground level was higher than it had been previously, varying from 16 to 25° C. with the maximum at 4 p.m. The temperature at 6 ft. and of the soil can be seen from the table.

Humidity. The humidity, fairly high at the com-

Table 6. 22 *May* 1947. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at 2-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	7·8	6·5	10·0	77	92	Nil	25	0	25	530
4 a.m.	7·8	6·0	10·0	84	87·5	40	296	4	300	465
6 a.m.	10·0	9·0	9·4	79	88	760	110	10	120	450
8 a.m.	14·4	14·0	11·1	64	78	Above 1000	108	12	120	260
10 a.m.	16·1	16·5	15·0	60·5	77	Above 1000	30	0	30	310
12 noon	14·7	15·0	17·2	64	71	Above 1000	40	0	40	166
2 p.m.	13·9	13·5	15·3	69·5	87	740	40	0	40	115
4 p.m.	15·0	14·5	15·6	64·5	78·5	800	39	6	45	67
6 p.m.	13·9	14·5	15·0	75·5	78·5	470	88	12	100	40
8 p.m.	12·8	13·1	14·4	77	87	35	56	4	60	20
10 p.m.	12·8	13·1	13·9	91	94	Nil	152	28	180	293
12 m.n.	12·8	13·5	13·9	98·5	99	Nil	344	16	360	435

Table 7. 28 *June* 1947. ***Numbers of infective larvae of*** **Haemonchus contortus** ***recovered from grass and surface layer of soil at 2-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.***

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	19·4	16·0	20·0	95	98·5	Nil	110	10	120	333
4 a.m.	19·7	16·0	16·9	90	98·5	90	318	12	330	160
6 a.m.	19·7	19·0	17·2	84	91·5	450	128	22	150	98
8 a.m.	22·2	21·0	18·9	71	85	Above 1000	92	15	107	104
10 a.m.	24·4	21·5	20·6	65	78	Above 1000	55	5	60	98
12 noon	24·4	21·0	21·7	65	90	Above 1000	55	8	63	57
2 p.m.	26·7	24·0	22·8	59	75	Above 1000	24	6	30	64
4 p.m.	27·2	25·0	23·3	55	78	Above 1000	26	4	30	54
6 p.m.	18·9	21·0	21·1	84	80	135	30	5	35	73
8 p.m.	18·3	23·0	20·0	89	69	75	65	15	80	72
10 p.m.	15·6	17·0	17·8	94	64	Nil	64	6	70	96
12 m.n.	16·1	20·0	17·2	87	56	Nil	30	2	32	84

mencement of the period, fell slightly with the rise of temperature during the day.

Light intensity. The duration of the period of daylight was similar to that in the previous month, but the intensity was greater, being above 1000 f.c. from 8 a.m. to 4 p.m.

Total number of larvae on grass. The rise of larvae on the grass was rapid, a maximum of 330 being recovered at 4 a.m., the earliest maximum so far. The number recovered then fell to a minimum of 30 at 2 and 4 p.m. and rose to an evening maximum at 8 p.m., which was not as high as the morning one. It is interesting to note that the two peaks on the curve are more widely separated here than at any other time during the year. The number of larvae on the bottom 1 cm. of grass was, in all cases, greater than the number higher up, but the two maxima were apparent in both regions.

Larvae in top 5 mm. of soil. The number recovered was high at the commencement of the period, fell during the day and rose again, slightly, towards the end.

Observation 8, 24 *July* 1947 (Table 8). A very fine rain fell almost throughout the 24 hr.

Temperature. The temperature at ground level was fairly high, varying from 13·5 to 17·5° C. The temperature at 6 ft. and of the soil can be seen from the table.

Humidity. The humidity was high, reaching saturation point at some times of the day. It was, on the whole, higher at ground level than at 6 ft.

Light intensity. There was again a long period of daylight, but the intensity was not as high as might be expected at this time of the year owing to the rain. The intensity did, however, exceed 1000 f.c. at 10 a.m. and at noon.

Total number of larvae on grass. The total number of larvae recovered from the grass was fairly low throughout the period. Nevertheless, two maxima were again apparent, a morning maximum at 4 a.m. and an evening maximum at 6 p.m. This was so in spite of the continuous fine rain. As Taylor (1938*b*) pointed out, comparatively few nematode larvae are washed off the herbage by light rain, though they may be washed off by heavy rain. The larvae of *H. contortus* do not move very rapidly in wet surroundings, which may account for the small number of larvae climbing. It is interesting to note that, when the humidity was relatively constant and very high, the same two maxima were observed during the day. This seems to indicate that humidity alone does not account for the morning and evening rise, and that light of low intensity is probably a more vital factor. In spite of the rain there was no rise in the hours of darkness as there was in May.

Larvae in top 5 mm. of soil. The numbers of larvae recovered from the surface layer of the soil was high throughout. They were again high at both ends of the period, but the fall during the middle of the day was not as marked as on any previous occasion, when the day had been relatively dry. Possibly there was not as great a migration into the deeper parts of the soil as conditions on the surface were favourable.

Observation 9, 19 *August* 1947 (Table 9). Very hot, sunny day.

Temperature. The temperature at ground level was higher than at any other time during the year, varying from 16·5° C. at 6 a.m. to 31° C. at noon. The

Table 8. 24 *July* 1947. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at 2-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total or 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	15·0	15·0	15·6	82	85	Nil	62	15	77	520
4 a.m.	14·4	13·5	15·6	59	90·5	15	112	20	132	344
6 a.m.	15·0	16·0	14·4	88	82·5	210	77	0	77	270
8 a.m.	17·2	17·0	15·6	83	92	940	62	15	77	220
10 a.m.	18·3	17·0	16·7	78·5	100	Above 1000	46	10	56	235
12 noon	18·3	16·5	17·2	78·5	100	Above 1000	46	10	56	235
2 p.m.	17·2	17·0	17·2	94	94	880	45	5	50	175
4 p.m.	17·2	17·5	17·2	88·5	91·5	920	50	5	55	270
6 p.m.	17·2	17·5	17·2	88·5	89	170	78	10	88	525
8 p.m.	16·1	17·5	17·2	94	86·5	35	60	15	75	530
10 p.m.	16·1	17·0	17·2	94	91·5	Nil	26	14	40	420
12 m.n.	16·1	17·0	17·2	94	91·5	Nil	73	14	87	400

Table 9. 19 *August* 1947. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at 2-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	20·0	20·5	18·9	70	70	Nil	69	0	69	355
4 a.m.	18·3	17·5	18·3	78·5	94	15	66	11	77	289
6 a.m.	17·8	16·5	17·8	78	84	105	83	21	104	288
8 a.m.	20·6	20·0	18·3	75	75	880	74	14	88	361
10 a.m.	25·0	24·5	23·9	53·4	50	Above 1000	54	6	60	340
12 noon	28·9	28·0	26·1	51	32·5	Above 1000	42	3	45	352
2 p.m.	30·0	31·0	31·7	39	16	Above 1000	36	2	38	338
4 p.m.	28·9	29·5	31·1	44	22	Above 1000	35	2	37	310
6 p.m.	26·1	27·0	29·4	58·9	46	720	35	3	38	325
8 p.m.	22·8	24·5	27·4	80	42	20	30	2	32	357
10 p.m.	18·9	19·5	28·8	79	69	Nil	17	0	17	328
12 m.n.	18·3	19·5	21·7	78·5	69	Nil	11	0	11	421

temperature at 6 ft. was, on the whole, fairly near that at ground level, and the temperature of the soil, in the second half of the day, higher.

Humidity. As the temperature rose during the day the humidity fell. It measured 94 % at ground level at 4 a.m. but had fallen to 16 % by 2 p.m., rising again to 69 % at 10 p.m. The humidity at 6 ft. was a little higher. The grass was quite dry and the surface of the soil hard and baked by the early afternoon.

Light intensity. There was again a long period of daylight and an intensity of over 1000 f.c. from 10 a.m. to 4 p.m.

Total number of larvae on grass. The total number of larvae recovered from the grass was on no occasion high. There was a rise in the early morning to a maximum of 104 at 6 a.m. Thereafter there was a gradual fall in the number, throughout the rest of the 24 hr. period, to a minimum of 11 at midnight. No evening maximum occurred. The majority of the larvae were recovered from the lower region of the grass, there being extremely few in the upper region especially from midday onwards. It is of course possible that some of the larvae, even though they succeed in climbing early in the day, were killed by the high temperatures and so were not recovered.

Monnig (1930) stated that infective nematode larvae do not recover after being dried and that some are killed by sunlight. Although conditions of light and temperature were favourable for an evening rise, it did not occur. This was probably due to the dryness of the grass and the surface of the soil. It seems evident, therefore, that larval migrations on to the grass are dependent on a combination of several essential factors.

Larvae in top 5 mm. of soil. The number of larvae recovered from the top 5 mm. of soil was high throughout the 24 hr., there being no marked fall during the middle of the day. In spite of the dryness of the soil and the high temperature they seem to have remained in the surface layer and to have survived.

Observation 10, 24 *September* 1947 (Table 10). Dull with occasional bright periods.

Temperature. The temperature shows a fall over the previous months, varying from 10° C. at 6 a.m. to a maximum of 16° C. at 10 a.m. The temperature at 6 ft. and of the soil can be seen from the table.

Humidity. The humidity of the air at ground level was fairly high, being lower during the middle of the day.

Light intensity. The duration of the period of daylight was shorter than in the previous month; the intensity of light exceeded 1000 f.c. from 10 a.m. to 2 p.m.

Total number of larvae on grass. Two maxima were once more apparent, the first at 8 a.m. and the second at 6 p.m., the numbers recovered were relatively high. The majority of the larvae were again found in the lower portions of the grass blades. It is interesting to note that the two peaks on the curve are approaching one another as the length of the period of daylight decreases.

Larvae in top 5 mm. of soil. Large numbers were again recovered at both ends of the period. There was a fall during the middle of the day.

Observation 11, 22 *October* 1947 (Table 11). Dull, damp day.

Temperature. The temperature at ground level varied from 11 to 14·5° C., the lower temperatures occurring late in the day. The temperature at 6 ft. was generally above that at ground level, and the temperature of the soil, below.

Humidity. The humidity of the air at ground level was fairly high and higher than that at 6 ft.

Light intensity. There was a further decrease in the duration of the period of daylight which lasted from 8 a.m. to 6 p.m. The intensity exceeded 1000 f.c. only at noon.

Total number of larvae on grass. The two maxima now occurred at 8 a.m. and 4 p.m., 427 larvae being recovered in the morning and 232 in the afternoon. The two peaks on the curve are even closer than in the last month, the length of daylight being less.

Larvae in top 5 mm. of soil. Larger numbers were recovered at both ends of the period but were relatively high all through. This may be because conditions of temperature and humidity were favourable to their remaining in the surface layer of the soil.

Table 10. 24 *September* 1947. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at 2-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	11·1	11·0	12·2	73	93	Nil	147	12	159	361
4 a.m.	11·1	11·0	12·2	73	93	Nil	141	9	150	340
6 a.m.	10·0	10·0	11·7	79	93	45	444	9	453	254
8 a.m.	12·2	10·5	11·1	68	86·5	720	225	3	228	179
10 a.m.	15·6	16·0	13·9	65	71·5	Above 1000	75	9	84	151
12 noon	15·0	15·0	13·9	64·5	73	Above 1000	81	6	87	153
2 p.m.	16·1	14·5	14·4	60·5	78·5	Above 1000	60	0	60	108
4 p.m.	14·4	13·5	14·4	64	87	840	54	0	54	60
6 p.m.	13·3	13·0	13·9	69	81	40	144	18	162	185
8 p.m.	12·2	12·0	13·3	74	93	Nil	14	9	123	188
10 p.m.	10·0	11·0	12·8	86	93	Nil	93	3	96	319
12 m.n.	10·0	11·0	11·7	86	93	Nil	90	12	102	317

Table 11. 22 *October* 1947. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at 2-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	12·8	12·0	11·1	56	61	Nil	166	3	169	539
4 a.m.	13·3	13·0	11·7	69	81	Nil	150	3	153	508
6 a.m.	13·3	12·5	12·2	75	87	Nil	163	3	166	618
8 a.m.	13·3	13·0	12·2	75	81	225	408	19	427	300
10 a.m.	13·3	13·0	12·2	81	90	190	125	3	128	404
12 noon	15·6	14·5	12·8	60	76	Above 1000	122	3	125	383
2 p.m.	13·3	12·5	12·8	75	77	620	125	3	128	395
4 p.m.	12·8	13·0	12·2	69	71	125	229	3	232	329
6 p.m.	11·7	11·0	12·2	74	86	10	144	5	149	315
8 p.m.	11·1	11·0	11·7	86	93	Nil	137	13	150	486
10 p.m.	11·1	11·0	11·7	86	86	Nil	159	2	161	378
12 m.n.	11·1	12·0	11·7	79	71	Nil	138	0	138	489

Table 12. 26 *November* 1947. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass and surface layer of soil at 2-hourly intervals, together with meteorological and micrometeorological data at the time of sampling.*

(Each plot infected with a total of 1200 larvae.)

Time (G.M.T.)	Temp. °C. at 6 ft.	Temp. °C. at ground level	Temp. °C. soil at depth of 2 in.	% R.H. at 6 ft.	% R.H. at ground level	Light intensity (f.c.)	No. of larvae on bottom 1 cm. of grass	No. of larvae on top 2 cm. of grass	Total no. of larvae on grass	No. of larvae in top 5 mm. soil
2 a.m.	5·0	5·0	4·4	57	82·5	Nil	64	0	64	520
4 a.m.	4·4	4·0	4·4	65	95	Nil	45	3	48	204
6 a.m.	4·4	3·5	4·4	56	91	Nil	40	3	43	224
8 a.m.	3·3	1·5	4·2	63	95	55	85	7	92	160
10 a.m.	3·9	2·0	3·3	55	90	340	36	4	40	156
12 noon	5·6	5·0	3·3	42	61	420	36	2	38	230
2 p.m.	5·6	3·5	4·2	54	91	720	32	2	34	250
4 p.m.	3·3	1·0	4·4	63	95	80	54	10	64	144
6 p.m.	2·8	0·5	3·9	71	95	Nil	50	6	56	87
8 p.m.	2·8	0·5	3·3	71	95	Nil	47	3	50	49
10 p.m.	0·0	−0·5	3·3	68	89	Nil	25	0	25	39
12 m.n.	−0·6	−0·5	3·3	78	89	Nil	31	0	31	48

Observation 12, 26 *November* 1947 (Table 12). Fine day, damp morning and evening.

Temperature. The temperature at ground level was low, varying from −0·5 to 5·0° C., the highest temperature occurring from midday to 2 p.m., and the lowest in the last few hours. The temperature of the soil was less variable, 3·3–4·4° C. The relationship between the various temperatures can be seen from the table.

Humidity. The humidity at ground level was higher than at 6 ft. This may be due to evaporation of moisture from the surface of the soil.

Light intensity. The duration of the light period was at a minimum, namely, from 8 a.m. to 4 p.m. On no occasion did the intensity exceed 1000 f.c., the condition being very similar to December 1946.

Total number of larvae on grass. The two maxima were at 8 a.m. and at 4 p.m., as in October. The numbers recovered were small, possibly owing to the low temperatures which may have rendered the larvae less active.

Larvae in top 5 mm. of soil. The numbers recovered from the soil were high at the commencement of the period. There was another rise at midday followed by a fairly rapid fall to the end of the period. The

rise at midday may have been due to the rise in temperature, and the subsequent fall to a lowering of the temperature to below freezing point.

DISCUSSION

The factors examined in relation to the vertical migrations of the infective larvae of *H. contortus* on the grass blades were: (1) temperature, (2) humidity and (3) light intensity. These factors will now be discussed in turn.

Temperature. The temperature at ground level varied throughout the year, being at a minimum in February and a maximum in August. Temperatures were, on the whole, low in the winter months, high in the summer months and moderate in the spring and autumn. The difference between the maximum and minimum temperatures for any particular day were, on the whole, less in the winter than the summer, varying from 2° C. in December to 13·5° C. in August (Fig. 1). The temperatures were, mainly, lowest at night and highest during the middle of the day, the rise and fall being fairly gradual.

Humidity. As humidity is closely bound up with temperature it is difficult to consider these factors separately. The humidity at ground level was lowest in February and August, owing presumably to the very low temperatures in the first instance and to the high temperatures in the second. Apart from these two occasions the humidity was in all cases fairly high, being highest in July when precipitation was almost continuous. During individual days the humidity was generally higher in the early morning and evening than in the middle of the day.

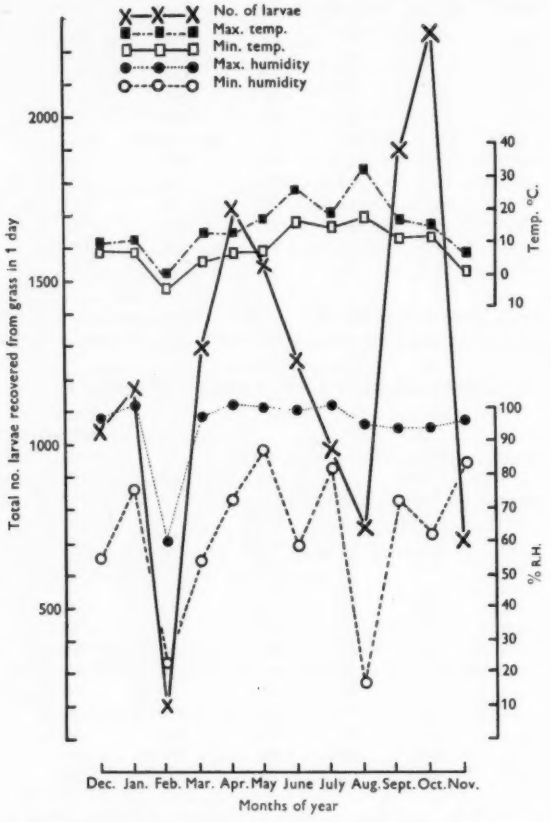

Fig. 1. Showing total number of larvae of *Haemonchus contortus* recovered from grass during each of the 12 days of observation. Maximum and minimum temperature and maximum and minimum percentage relative humidity, at ground level, also figured.

In Table 13 and Fig. 1 the total number of larvae recovered from the grass for each of the 12 days of

Table 13. *Total number of infective larvae of* Haemonchus contortus *recovered from grass on each of the 12 days of observation, together with maximum and minimum temperature and percentage relative humidity, at ground level, for each day*

Date	Total no. of larvae on grass	Temperature at ground level (°C.)		% R.H. at ground level	
		Maximum	Minimum	Maximum	Minimum
2 Dec. 1946	950	8·5	6·5	96	53
11 Jan. 1947	1048	10·0	6·0	Saturation	74
27 Feb. 1947	93	−1·0	−5·5	58	22
25 Mar. 1947	1163	12·2	2·5	96·5	52·5
29 Apr. 1947	1597	11·1	5·8	Saturation	72
22 May 1947	1420	16·5	6·0	99	87
28 June 1947	1107	25·0	16·0	98·5	56
24 July 1947	870	17·5	13·5	Saturation	82·5
19 Aug. 1947	616	31·0	16·5	94	16
24 Sept. 1947	1758	16·0	10·0	93	71·5
22 Oct. 1947	2126	14·5	11·0	93	61
26 Nov. 1947	585	5·0	−0·5	95	82·5

observation throughout the year is shown, together with the maximum and minimum temperature and the maximum and minimum percentage relative humidity. It will be seen that the maximum numbers were obtained in the spring and autumn, namely, March, April, May, and September and October. This seems to correspond with the temperature and humidity. There was a decided fall in the number recovered in February when it was very cold and dry, and in August when it was very hot and dry. Even though the humidity is high during the winter months the low temperature seems to inhibit upward vertical migrations. In July, when the humidity was at its highest, the larvae did not climb well even though the temperature was favourable. It seems that the two extremes of very high and very low humidity and very high and very low temperatures inhibit movement. A certain range of both temperature and humidity combined is evidently necessary for a maximum amount of migration up the grass blades, the optimum conditions for both these factors, in this instance, occurred in spring and autumn, showing a rise in the total number of larvae recovered from the grass blades at these times. From this it would seem that sheep are most likely to pick up more larvae in the spring and autumn than in midsummer and midwinter, but it is difficult to generalize from observations made on only one day in each month of the year. Leiper (1928) has suggested that sheep should do well during a dry summer because of the destructive effect of sunlight on the larvae. Hawkins (1945), too, has pointed out that infection of sheep with strongyles varies with temperature and moisture. He is of the opinion that spring infections are partially thrown off unless a wet summer causes heavy reinfestation. Buckley (1940), in his laboratory experiments on the larvae of *H. contortus*, found that temperature was an important factor in determining vertical migrations.

The total numbers of larvae recovered from the top 5 mm. of soil for each day of observation were as follows: December 1275, January 1387, February 140, March 1685, April 1871, May 3151, June 1293, July 4144, August 4014, September 2615, October 5144, November 2111. These figures are not represented on the graph. The low number recorded for February may have been due to freezing and death of the larvae or to their migration to a greater depth. The number remaining in the surface layer of the soil was high in July, when a fine rain fell continuously throughout the day. Conditions were then probably favourable to the larvae and there would be no stimulus to move away from them. It is surprising that in spite of the dryness of the soil in August a large number of larvae was recovered. As Kauzal (1941) has pointed out, the soil may serve as a reservoir for the larvae where they may survive for a long period and from which contamination of the foliage can take place in suitable conditions of temperature and humidity.

Light. From the twelve observations described in the preceding pages, it seems that, provided temperature and humidity are favourable, light is of great importance in determining the time of day when the maximum number of larvae are on the grass blades. The length of the light period varied throughout the year, being shorter in the winter and longer in the summer, as can be seen from Table 14. The variation in the intensity of the light throughout the year, therefore, was greatest in the early morning and in the evening. The times at which morning and evening maxima occurred in each month are included in the table. In the winter months sunrise is later, the period of dim light occurring later in the day than in the summer months when sunrise is earlier. Passing from the winter to the summer months, therefore, the morning maximum became progressively earlier. In December and January it occurred at 10 a.m., in March and April at 6 a.m., and in May to July at 4 a.m. Thereafter, in the

Table 14. *Duration of light period on one day for each month of the year and times of recovery of maximum numbers of larvae from grass*

Month	Duration of light period	Time (G.M.T.) of morning maximum	Time (G.M.T.) of evening maximum
Dec. 1946	8 a.m.–4 p.m.	10 a.m.	4 p.m.
Jan. 1947	8 a.m.–4 p.m.	10 a.m.	4 p.m.
Feb. 1947	8 a.m.–6 p.m.	No apparent maximum	No apparent maximum
Mar. 1947	6 a.m.–6 p.m.	6 a.m.	6 p.m.
Apr. 1947	4 a.m.–8 p.m.	6 a.m.	8 p.m.
May 1947	4 a.m.–8 p.m.	4 a.m.	8 p.m.
June 1947	4 a.m.–8 p.m.	4 a.m.	8 p.m.
July 1947	4 a.m.–8 p.m.	4 a.m.	6 p.m.
Aug. 1947	6 a.m.–8 p.m.	6 a.m.	No evening maximum
Sept. 1947	6 a.m.–6 p.m.	6 a.m.	6 p.m.
Oct. 1947	8 a.m.–6 p.m.	8 a.m.	4 p.m.
Nov. 1947	8 a.m.–4 p.m.	8 a.m.	4 p.m.

Table 15. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass at 2-hourly intervals on five selected days throughout the year*

(Morning and evening maxima in heavy type.)

	Nos. of larvae recovered from grass at 2-hourly intervals											
	a.m.						p.m.					
Date	2	4	6	8	10	Noon	2	4	6	8	10	M.n.
2 Dec. 1946	32	50	54	139	**196**	74	80	**120**	69	69	50	17
25 Mar. 1947	55	94	**155**	135	124	90	50	70	**135**	101	90	60
28 June 1947	120	**330**	150	107	60	63	30	30	35	**80**	70	32
24 Sept. 1947	159	150	**453**	228	84	87	60	54	**162**	123	96	102
22 Oct. 1947	169	153	166	**427**	128	125	128	**232**	149	150	161	138

second half of the year it became successively later, occurring at 6 a.m. in August and September and at 8 a.m. in October and November. Similarly, the evening maximum moved to a later time in the summer months. In December and January it occurred at 4 p.m., in March at 6 p.m., and in April to June at 8 p.m. In the second half of the year the evening maximum became progressively earlier again. Morning and evening maxima did not occur in February, and the evening maximum was not present in August owing to unfavourable conditions of temperature and humidity. Five selected days throughout the year have been taken to show the varying positions of the two maxima (Table 15, Fig. 2). The two peaks on the curves move away from one another as the light period becomes longer and approach one another as it becomes shorter. This seems to indicate that the infective larvae of *H. contortus* respond most readily to light of comparatively low intensities, a condition which obtains shortly after sunrise and before sunset. The response will, of course, depend on other conditions being favourable.

If the results are considered for the time of day throughout the year (Table 16, Fig. 3), it will be seen that at 4 a.m. there are more larvae on the grass in May and June than in the other months, at 6 a.m. the greatest number was recorded in April and September, at 8 a.m. in January and October, and at 10 a.m. in December, January and October. As regards the evening maxima more were present on the grass at 4 p.m. in December, January and October, at 6 p.m. in April and September, at 8 p.m. in April and October, and at 10 p.m. in May and October. Here the total numbers of larvae recovered at the different times varied more than in the morning and therefore the same trend is not so easily seen.

It seems, therefore, that the vertical migration of *H. contortus* larvae on grass is very largely due to the stimulus of light of a particular intensity or within a certain range. The light intensities measured at the time of maximum recovery were low, varying from 15 to 560 f.c. Of the total of twenty-one morning and evening maxima, seventeen occurred at an intensity of below 200 f.c., two between 200–300 f.c., and the remaining two at 470 and 560 f.c. respectively.

Light of a particular intensity alone was not sufficient to induce vertical movements as is seen in February and August, where, though the light was favourable, conditions of temperature and humidity were not. From a consideration of all factors examined, it seems that a favourable combination

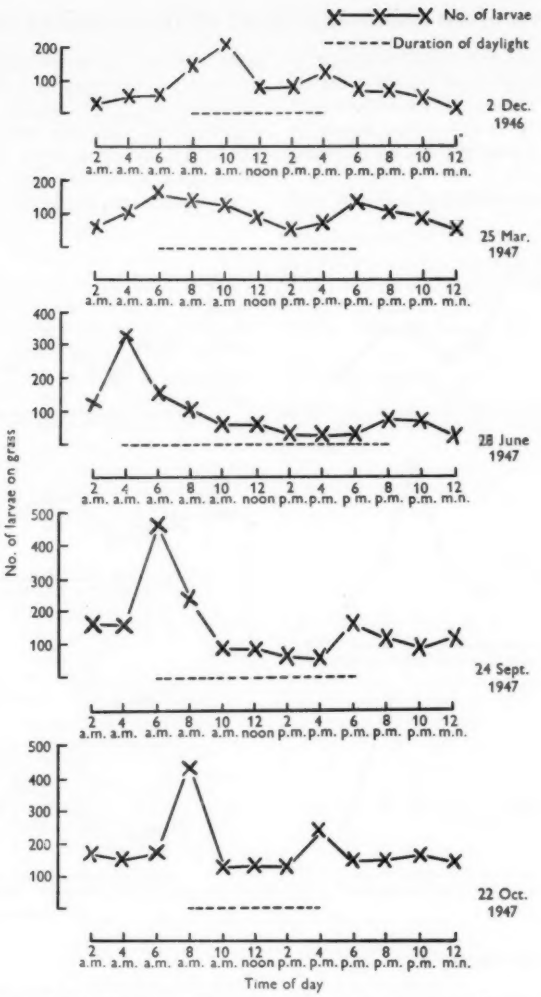
No. of larvae
Duration of daylight
No. of larvae on grass
2 Dec. 1946
25 Mar. 1947
28 June 1947
24 Sept. 1947
22 Oct. 1947
2 a.m. 4 a.m. 6 a.m. 8 a.m. 10 a.m. noon 2 p.m. 4 p.m. 6 p.m. 8 p.m. 10 p.m. 12 m.n.
Time of day

Fig. 2. Showing numbers of larvae of *Haemonchus contortus* recovered from grass at 2-hourly intervals on five selected days throughout the year.

Table 16. *Numbers of infective larvae of* Haemonchus contortus *recovered from grass from* 4 *to* 10 *a.m. and from* 4 *to* 10 *p.m. on each of the* 12 *days of observation*

	Date											
Time of day (G.M.T.)	2 Dec. 1946	11 Jan. 1947	27 Feb. 1947	25 Mar. 1947	29 Apr. 1947	22 May 1947	28 June 1947	24 July 1947	19 Aug. 1947	24 Sept. 1947	22 Oct. 1947	26 Nov. 1947
4 a.m.	50	40	0	94	120	300	330	132	77	150	153	48
6 a.m.	54	30	15	155	226	120	150	77	104	453	166	43
8 a.m.	139	185	7	135	144	120	107	77	88	228	427	92
10 a.m.	196	200	0	124	99	30	60	56	60	84	128	40
4 p.m.	120	116	8	70	138	45	30	55	37	54	232	64
6 p.m.	69	35	12	135	146	100	35	88	38	162	149	56
8 p.m.	69	40	10	101	172	60	80	75	32	123	150	50
10 p.m.	50	32	6	90	130	180	70	40	17	96	161	25

of temperature, humidity and light intensity is necessary for vertical migration of infective larvae of *H. contortus* on grass blades. Of these factors it seems that light intensity is the prime factor in determining the time of day at which the maximum amount of migration occurs.

In all cases the evening maximum is not as well marked as the morning, even though the light intensity, temperature and humidity may be similar at both times. It is possible that the larvae may be less responsive after a period of exposure to light than to light following darkness, or there may be a difference in the quality or wave-length of the light, at both ends of the day, which may induce a slightly lesser response in the evening than in the morning; a decrease in humidity and increase in temperature, which usually takes place during the middle of the day, may render the larvae less active. These are circumstances which will have to be determined by further experiments.

It is proposed, therefore, to follow up these preliminary observations by experiments in the laboratory when each factor can be investigated separately and it can be determined what combination of temperature, humidity and light intensity produce the maximum amount of vertical migration up the grass blades.

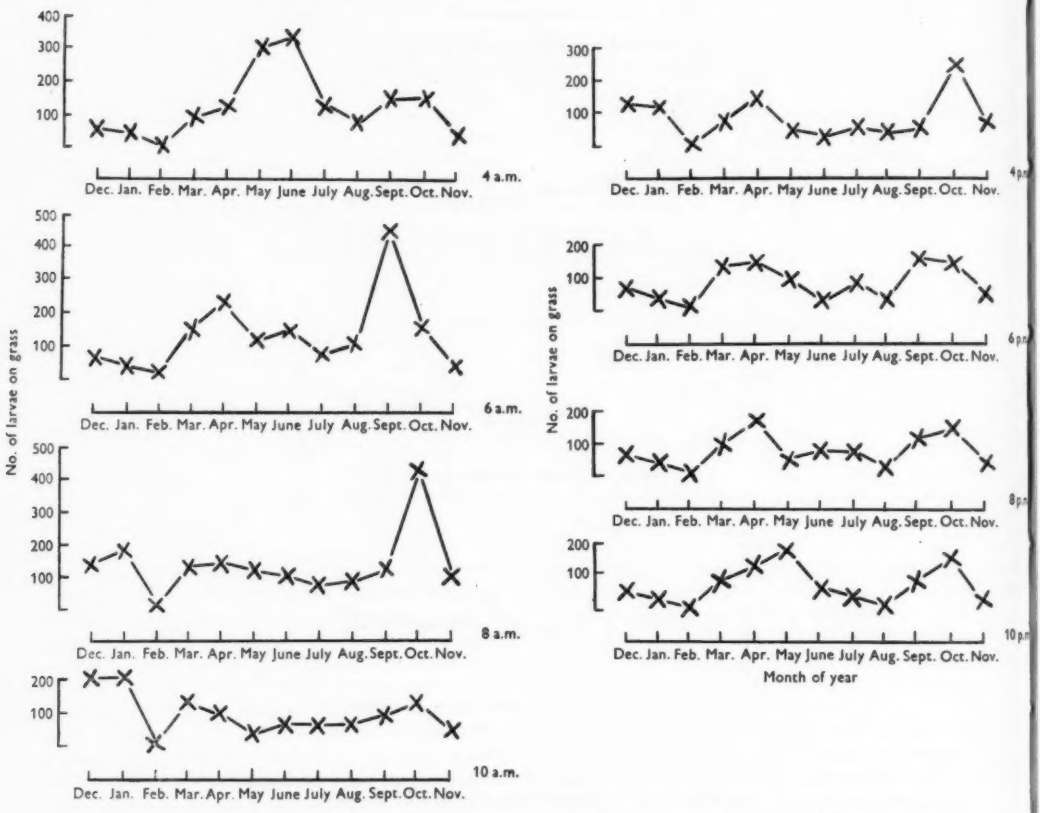

Fig. 3. Showing numbers of larvae of *Haemonchus contortus* recovered from grass from 4 to 10 a.m. and from 4 to 10 p.m. on each of the 12 days of observation.

SUMMARY

1. The cycle of vertical migration of the infective larvae of *Haemonchus contortus* on experimental plots of *Lolium perenne* S 24, kept out of doors, has been related to meteorological and micrometeorological factors.

2. The three main factors concerned with vertical migration are temperature, humidity and light intensity.

3. In the majority of cases the greatest number of larvae are on the grass blades in the early morning and in the evening, less being recovered at night and during the middle of the day. The time of the morning maximum becomes progressively earlier passing from winter to summer, and the time of the evening maximum progressively later. The reverse is true in the second half of the year.

4. Low and high temperatures accompanied by low humidity inhibit vertical migration on the grass.

5. High humidity (continuous precipitation) does not prevent migration, the two daily maxima are apparent, but the numbers climbing are less than in the absence of rain.

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ANOPHELINE LARVAE AS INTERMEDIATE HOSTS FOR LARVAL TREMATODES

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(With 2 Figures in the Text)

INTRODUCTION

The literature on mosquito larvae as intermediate hosts of larval Trematodes is scanty. Porter (1938) states that it is well known that anopheline larvae act as intermediate hosts for Xiphidiocercaria, but she cites no instances. Harper (1929) experimentally infected twenty-five larvae of *Culex pipiens* with the Xiphidiocercaria, X.I., whilst Eckstein (1922) and Ruge (1903) record finding the encysted stage in mosquito larvae.

In all cases that the writer has encountered in the literature on this subject the encysted stage is always referred to, yet while carrying out a survey in Tissamaharrama, South-Eastern Ceylon, a large number of anopheline larvae were found infected with cercariae; but no cysts were evident. The following contribution, therefore, deals with an account of this cercaria, together with an indication of its possible life history.

TOPOGRAPHY OF TISSAMAHARRAMA

Tissamaharrama is in the dry zone of South-Eastern Ceylon, having a very low rainfall. It is a typical rice-producing area. The district is flat and has a number of wewas or tanks which serve as reservoirs collecting water in the rainy season from the river Kirinda Oya by means of channels. There are five tanks, three of which: Debara wewa, Yoda Kandiya wewa and Wirawila wewa are connected directly to the Kirinda Oya. Of the remaining two, Tissa wewa is connected to Yoda wewa, which in turn is in communication with the river. The water necessary for the successful cultivation of rice is supplied from these tanks by means of main irrigation channels, which in turn divide into subsidiary and tertiary channels supplying the paddy fields.

This investigation was carried out in the dry season, the tanks were at low ebb owing to the continual feeding of the paddy fields and the channel between Tissa wewa and Yoda wewa was more or less dry, a few pools remaining fed by a leak from Tissa wewa.

SPECIES OF ANOPHELINES INFECTED

The time at the writer's disposal was extremely limited, yet as many anopheline larvae from as wide an area as possible were examined for infection, but only three species were found to harbour the parasite, *Anopheles culicifacies*, *A. aconitus* and *A. annularis*, with the following percentage infection:

A. aconitus	68 %
A. culicifacies	40 %
A. annularis	12 %

These percentages were drawn from the examination of many hundreds of larvae of the three species. The infected larvae were found in the Yoda Kandiya wewa, Tissa wewa, Debara wewa and some of the subsidiary channels, but the heaviest infection was encountered in larvae collected from the channel between Tissa wewa and Yoda wewa. The distribution of infection is shown in the map (Fig. 1).

TECHNIQUE

The writer is definitely of the opinion that some degree of standardization should be sought for the study of larval Trematodes. Many workers have introduced new species on the basis of size difference, yet the method used for measurement differs with the various workers. It is well known that different reagents have different effects upon the cercarial stage, some becoming swollen, others contracting to a marked degree. It is maintained, therefore, that all measurements should be carried out on the living material, but again some uniformity of method is essential. Cercaria will expand and contract more under heavy pressure due to excessive withdrawal of water from under the cover-slip than if the slide remains flooded.

The advantages of the study of the living material was clearly pointed out by Looss (1894). He and later investigators showed that the excretory system can be best observed with living cercaria. Since, as pointed out by Faust (1922), 'no organs of the cercaria save the cephalic glands and the flame-cell

pattern have thus proved satisfactory for the differential diagnosis' the writer has carried out his studies on the living material. Measurements were made in water under a cover-slip, water being withdrawn by means of a piece of filter-paper until active movement of the cercaria just ceased. The gland-cell pattern was determined by staining with a solution of neutral red.

LOCATION OF THE PARASITE IN THE ANOPHELINE BODY

There was no trace of sporocyst or redia, only the cercarial stage was found. Most of the cercariae were encountered in the body cavity of the thorax, in some specimens eleven to twelve parasites were counted, the average, however, was about four to

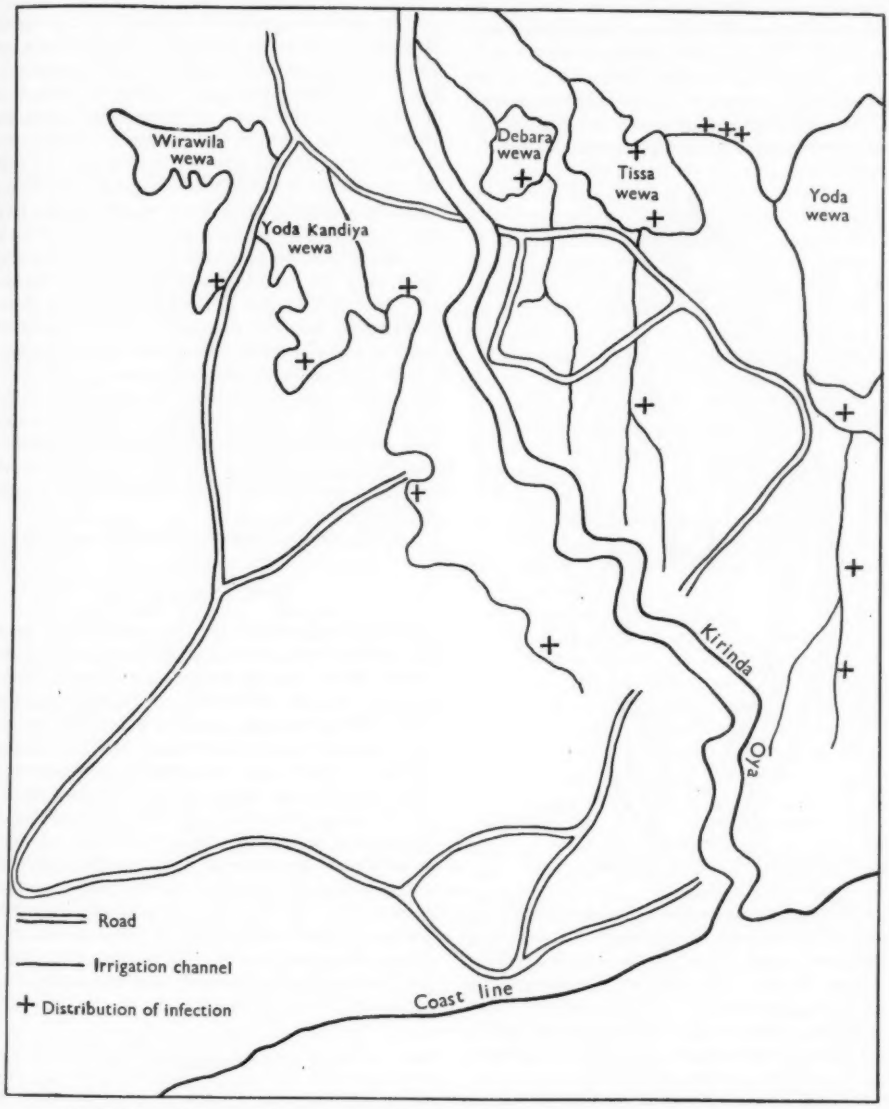

Fig. 1. Map of Tissamaharrama, South-Eastern Ceylon, showing distribution of *Cercaria anophelini* infection in anopheline larvae.

five. A smaller number were seen in the second and third abdominal segments, the average here being between one and two. No parasites were found in any of the segments posterior to the third abdominal.

STRUCTURE AND ANATOMY OF THE CERCARIA

In all the cercariae examined a tail was absent. The cercariae were extremely active, moving about with characteristic worm-like expansions and contractions. The body length varies from 245 μ in the fully expanded condition to 100 μ fully contracted, with a breadth varying from 96 to 30 μ in the expanded and contracted conditions respectively.

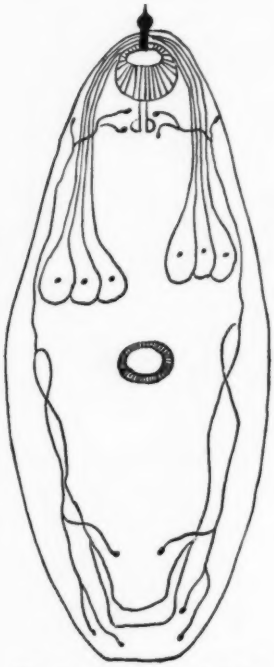

Fig. 2. *Cercaria anophelini.*

The oral sucker is larger than the acetabulum and is rounded to elongate according to its degree of movement and contraction. In its normal condition it measures 21–24 μ. The stylet, situated in the dorsal wall, is simple with a total length of 7–8 μ. It is sharply pointed and has a well-developed shoulder. The breadth at the base, the widest part, is 2–3 μ.

The acetabulum is muscular and situated just posterior to the middle of the body. It is devoid of spines and measures 14–15 μ.

The mouth opens on the oral sucker, and leads through a narrow prepharynx into a muscular pharynx. Neither oesophagus nor caeca were seen.

There are three salivary, or penetration, gland cells on each side of the body, lateral and slightly anterior to the ventral sucker. They are highly granular and have well-marked nuclei. The granular ducts from each pass forward and open separately on each side of the stylet.

The excretory system consists of a posteriorly disposed bladder or excretory vesicle which has a median chamber and a pair of lateral cornua, from each of which the main excretory vessels pass anteriorly to the level of the ventral sucker where division into anterior and posterior tubules occurs. The anterior tubule passes forward and at the level of the posterior margin of the pharynx bifurcates into two capillaries, one of which drains from a flame cell at the edge of the body on a level with the middle of the pharynx, while the other divides into two, one of which terminates in a flame cell at the level of the posterior margin of the oral sucker and the other in a flame cell near the anterior edge of the pharynx. The posterior collecting-tube passes posteriorly to the region of the cornua where it divides into two capillaries, one passing to the mid-line of the body where it terminates in a flame cell, while the other capillary continues posteriorly and bifurcates into two capillaries, each of which ends in a flame cell. The excretory formula is therefor 2 (3+3).

There was no trace of the genital system.

NOMENCLATURE

The whole question of the nomenclature of cercariae is a difficult one. Sewell (1922) is opposed to giving larval forms specific names, yet Faust (1924) has pointed out the difficulties of designating a larval form with a numeral, since later workers describing the same specimen may redesignate it with a literal system of their own, thus adding to the confusion. The writer is not aware of any cercariae described from Ceylon, hence he could follow Sewell's example by naming it *Cercaria ceylon* I. However, when it is possible to give a name which is descriptive of the larva itself or is indicative of any particular host, it is believed that this procedure should be adopted. Hence, in spite of the fact that this cercaria is not being described from its first intermediate host, the writer has, for the sake of easy reference, decided to call it *C. anophelini.*

CLASSIFICATION

Lühe (1909) defined the Xiphidiocercaria as a Distome group in which there was a slender tail and a boring spine. Later investigators have shown

that while the presence of a boring spine or stylet is a characteristic feature of the Xiphidiocercaria it is not limited to that group, for a stylet occurs in some Microcercous, Rhopalocercous and Cystocercous cercaria. The stylet of the Xiphidiocercaria is simple in structure, whereas in the other it is more complex. Lühe recognized four groups of stylet cercaria, but Sewell (1922) reclassified the Xiphidiocercaria, creating a number of well-defined smaller groups. Among his six groups of the Microcotylae, Sewell has the Pusilla group, to which the characters of the internal anatomy of *C. anophelini* fits perfectly. I have, therefore, placed this cercaria in the Pusilla group.

DISCUSSION

A large number of anopheline larvae were examined, but no redia or sporocysts were observed, and all the cercariae were tailless. It is obvious, therefore, that the anopheline larvae are the second intermediate host for this Trematode, the tail being shed after it had served its function of propelling the larva to its host, after which leech-like movements begin until the point of entry is reached.

In spite of the examination of many anopheline larvae no cysts were seen. In other records of mosquito larvae as intermediate hosts for larval Trematodes the encysted stage is always referred to, for example, Ruge (1903) speaks of the encysted cercaria from gnats, and Eckstein (1922) observes that the cysts are present in the last four abdominal segments of *Culex pipiens*. All authorities are agreed that encystment takes place immediately the larva penetrates its host. Harper (1929) remarks in his observations of the encystment of Cercaria X.I. into *Gammarus pulex* 'I have not observed the actual process of encystment in *Gammarus* (owing to the opacity in the body) but that it takes place very quickly is shown by the following: a *Gammarus* placed for 5 min. in close contact with many active cercariae provided on dissection 20 min. later 5 cysts from the tissues of the hepatopancreatic tubes'. The only exception to the immediate encystment of the stylet cercariae after penetration which the writer has met in literature is that of the cercaria of *Lecithodendrium chilostomum* (Mehl). Brown (1933) remarks that the cercaria of *L. chilostomum* does not encyst, as is usual for Xiphidiocercaria, immediately on penetrating its second intermediate host, the caddis-fly larva, but after the stylet is shed and the salivary glands disappear it lives freely in the body cavity as a metacercaria. It is probable, therefore, that encystment does not take place in the larvae in the case of *Cercaria anophelini* and the continued presence of the stylet and the penetration glands is also indicative that their functions are not finished.

The author was unable to carry out further work on the life history of this trematode, but suggests that the continued development occurs in one of two ways. In the first place, encystment may take place in those animals which are the natural enemies of anopheline larvae. A. A. Shandosham, in his *Malaria in Malai*, states that the natural enemies of mosquito larvae, other than fish, can be dismissed with a few words, the only efficient predator being the large *Belastomid* bug. However, Hem Singh Pruthi (1929) lists a wide range of natural enemies of mosquito larvae as a result of some experiments which he conducted. These include the fish, particularly the genus *Gambusia*, which is often bred for the purposes of biological control of the malaria-carrying mosquito; several members of the Hemiptera which pierce the larval body with their fine proboscis and suck out the body contents; the Coleopteran dytiscid larvae which pierce the larval body and suck out the fluids by means of long sickle-shaped and hollow mandibles; and many members of the Crustacea, among which the water-shrimps of the genus *Palaemon* may be cited, eat the mosquito larva whole. In all these cases the mosquito eventually arrives in the stomach of its predator, and, if the future development of *Cercaria anophelini* does take place in this manner, the presence of the stylet and the penetration glands would still be needed for penetration of the stomach wall and for progress to the place of encystment. Experiments carried out by various investigators have shown that cercariae can live in the gastric fluids of different hosts for at least an hour, which would be time enough for them to penetrate the stomach wall.

The author believes that encystment occurs in the adult mosquito, probably during the short pupation period. That such a course is possible is evidenced by the work of Brown (1933) on the life history of *Lecithodendrium chilostomum*. The cercaria enters the caddis-fly larva, lives for a period as a free metacercaria in the abdominal cavity, and then migrates to the thoracic region of the same host during pupation. Here encystment takes place and is carried forward to the imago upon which the final host, the bat, feeds. It is interesting to note that in the case of *Cercaria anophelini* the great majority of the cercariae were found in the thorax, and it is suggested that those found in the abdominal region will eventually make their way to the thorax. During the metamorphosis to the adult in the pupation period, it is further suggested that the stylet and penetration gland cells probably function in penetrating the muscles of the adult mosquito.

If this is the course of development, we should expect to find the adult fluke in a winged animal, either the mammalian bat or a bird, possibly of the swallow family.

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SOME OBSERVATIONS UPON PROLONGED TREATMENT OF *PLASMODIUM GALLINACEUM* INFECTION IN CHICKS WITH ANTIMALARIAL DRUGS

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I. INTRODUCTION

James & Tate (1937*a*, *b*, 1938) first showed that whereas infections of *Plasmodium gallinaceum* in the peripheral blood of chicks could be cured temporarily by quinine, the development of the exo-erythrocytic parasites was unaffected by the drug, and death occurred later from the occlusion of the capillaries of the brain by large schizonts in the endothelial cells lining the vessels. Brumpt, Bovet & Brumpt (1937) found that plasmoquine, rhodoquine and atebrin had a similar effect. More recently, infections of *P. gallinaceum* have been used extensively both in the United States and in this country as a standard method for determining the antimalarial activity of new synthetic compounds (Curd, Davey & Rose, 1945). In these tests an acute blood infection of short duration is used, in order that the results should not be complicated by the presence of exo-erythrocytic parasites, which are rare in the early stages of blood-inoculated infections and develop slowly.

The observations recorded in this paper deal with prolonged treatment of infections of *P. gallinaceum* in chicks, the objective being to increase our knowledge of the relationship of the exo-erythrocytic and erythrocytic cycles of development.

II. METHODS

In the experiments described in the following pages chicks ranging in weight from 40 to 80 g. were used. Unless otherwise stated, the infection was transmitted by the inoculation of parasitized blood diluted in citrate-saline solution into the pectoral muscle. The number of parasites in the inoculum was not determined, but all birds of a series received equal quantities (0·5 c.c.) of the same dilution of blood. In intravenous inoculation blood was injected into the jugular vein.

The drugs used were pamaquin (plasmoquine), mepacrine hydrochloride (atebrin), and quinine hydrochloride. They were administered in aqueous solution, by catheter tube, into the stomach of the bird. The doses were calculated in mg./20 g. of body weight of bird, and were adjusted daily to the body weight.

III. THE EFFECT OF THE STAGE OF DEVELOPMENT OF INFECTION WITH *PLASMODIUM GALLINACEUM* IN THE DONOR BIRD UPON THE COURSE OF INFECTION IN THE RECIPIENT

Adler & Tchernomoretz (1941) found that when birds, inoculated with *P. gallinaceum*, were treated daily with doses of 100–150 mg. of quinine per kg. of body weight the development of erythrocytic parasites was inhibited, but uninucleate parasites, derived from the unaffected exo-erythrocytic parasites, appeared in the erythrocytes of the peripheral blood. These uninucleate parasites failed to develop further unless the dose of drug was reduced. The presence of such uninucleate forms, in birds undergoing treatment with drugs in doses large enough to suppress development of the parasites in the red blood corpuscles, is an indication that exo-erythrocytic parasites are present in the internal organs of the host.

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It was observed that when birds, inoculated intramuscularly with blood infected with *P. gallinaceum*, were treated daily from the day of inoculation with the maximum dose of pamaquin, mepacrine, or quinine which the birds would tolerate, uninucleate parasites appeared in the peripheral blood, but the time of their appearance varied considerably in different birds. These uninucleate parasites failed to develop further, so long as drug treatment was continued. In birds inoculated with blood from normal, untreated donors in which the infection, though heavy, was still on the upgrade, the incubation period was relatively long, uninucleate parasites first being seen about the 18th day or later. When the inoculum consisted of blood taken from a bird undergoing treatment with the maximum tolerated dose of one of the above drugs, and in whose erythrocytes only uninucleate parasites were found, the incubation period in the drug-treated recipients was short, uninucleate parasites being found on the 7th–12th days.

These observations suggested that uninucleate parasites which arise from exo-erythrocytic schizonts and appear in the peripheral blood during treatment with drug in suppressive doses, produce a more intense and more rapid development of exo-erythrocytic parasites in new birds than do parasites whose normal development in the erythrocytes has not been inhibited by the action of drugs. To prove whether this supposition was correct the following experiment was performed.

A chick inoculated with parasitized blood was treated twice daily, from the day of inoculation, with doses of quinine (0·64 mg./20 g.) sufficient to suppress the development of the parasites in the erythrocytes. Uninucleate parasites were first seen in the peripheral blood on the 17th day; and on the 20th, when they had become numerous, the bird was bled from the jugular vein. A post-mortem examination made immediately after the blood had been withdrawn showed that the bird was heavily infected with exo-erythrocytic parasites. Part of the blood was diluted 1 : 10 in citrate-saline solution and inoculated intramuscularly (0·5 c.c. per bird) into seven normal chicks. Five of the birds (Table 1, Exp. A) were treated daily with doses of quinine, mepacrine, or pamaquin sufficient to suppress the development of parasites in the erythrocytes, the first dose being given immediately after inoculation. Uninucleate parasites appeared in the peripheral blood on the 9th–12th days. From the remainder of the blood 1·0 c.c. was mixed with a little heparin to prevent coagulation and injected intravenously into a normal chick. As the recipient received no drug the uninucleate parasites introduced were able to continue their development in the erythrocytes: within 24 hr. normal parasites in all stages of development were found in the peripheral blood. The bird was then bled and a dilution (1 : 10) of the blood in citrate-saline solution was inoculated (0·5 c.c. per bird) intramuscularly into six normal chicks. Four of the chicks were treated daily, from the day of inoculation, with drugs in the same dosage (Table 1, Exp. B) as in the former experiment. If the results of the two experiments are compared, it will be seen that whereas the incubation period in both sets of untreated control birds was similar, it was much shorter in the group of drugged birds inoculated with uninucleate parasites taken direct from the original donor (Table 1, Exp. A) than in the group inoculated with parasites from a similar source, but which had undergone development for 24 hr. in a normal bird (Table 1, Exp. B).

Table 1. *The effect of drugs upon infections of* Plasmodium gallinaceum *induced by inocula from donors heavily infected with exo-erythrocytic parasites before* (*Exps. A and C*) *and after* (*Exps. B and D*) *passage for* 24 *hr. through normal chicks*

Drug	Dose	Average day of appearance of parasites in blood
	Exp. A	
Quinine	10·24	10 (2)
Mepacrine	5·12	9 (1)
Pamaquin	0·64	11·5 (2)
Controls	—	5 (2)
	Exp. B	
Quinine	10·24	Negative till 28th day (1)
Mepacrine	5·12	21 (1)
Pamaquin	0·64	22 (2)
Controls	—	5 (2)
	Exp. C	
Quinine	10·24	7 (2)
Mepacrine	5·12	6 (1)
Control	—	5 (1)
	Exp. D	
Quinine	10·24	20 (2)
Mepacrine	5·12	23 (2)
Control	—	5 (1)

The dose of drug is calculated in mg./20 g. of body weight.

The number of chicks in each experiment is given in parentheses.

It seemed improbable that the more rapid and intense development of exo-erythrocytic parasites in Exp. A was due to an intrinsic change in the parasites of the inoculum resulting from their previous contact with the drug, as any such change would hardly have disappeared after a 24 hr. sojourn in an untreated bird. A more probable explanation is that parasites arising directly from exo-erythrocytic schizonts give rise to a more intense exo-erythrocytic

infection than that produced by parasites undergoing normal development in the erythrocytes.

To test this hypothesis experiments were performed similar to those described above, but with parasites from a sporozoite-inoculated bird which had received no drug. Blood containing parasites in all stages of development was removed from the jugular vein on the 8th day following infection. A post-mortem examination made immediately after withdrawal of the blood showed an intense exo-erythrocytic infection of the capillaries of the brain. A group of chicks was inoculated, as in Exp. A, with equal quantities of the blood mixed with citrate-saline solution and treated daily with suppressive doses of drug (Table 1, Exp. C). Of the parasitized blood 1·0 c.c. was passaged, by intravenous injection, through a normal bird, as in the former experiments. After 24 hr. the bird was bled and a dilution (1 : 10) of the blood in citrate-saline solution was inoculated in equal quantities into a group of birds which received similar suppressive drug treatment daily (Table 1, Exp. D). As in Exps. A and B, the time of appearance of uninucleate parasites in the peripheral blood of birds undergoing treatment with drug was much earlier in birds inoculated with blood taken directly from the donor (Table 1, Exp. C), than in those receiving similar drug treatment (Table 1, Exp. D), but inoculated with parasitized blood from the same source after a 24 hr. sojourn in a normal bird.

A careful examination of the internal organs of chicks through which the parasites were passaged for 24 hr. failed to reveal any exo-erythrocytic parasites after this period of development.

The results of these experiments, therefore, suggest that the wide variation in the incubation period, as determined by the appearance of uninucleate parasites, in birds undergoing suppressive treatment with pamaquin, mepacrine or quinine, is correlated with the intensity of infection with exo-erythrocytic parasites in the donor. Parasitized blood from a donor heavily infected with exo-erythrocytic parasites rapidly gives rise to an intense infection of exo-erythrocytic parasites, whereas parasitized blood from a donor in which no exo-erythrocytic forms can be found produces infections in which exo-erythrocytic parasites develop slowly. An intravenous passage of 24 hr. duration through a normal bird is sufficient to change an inoculum from a type giving rise to infections characterized by early development of exo-erythrocytic schizonts into one characterized by the late development of such forms.

This explanation was supported by further observations (Tables 2 and 3). In one series of experiments (Table 2) seventy-four chicks were inoculated intramuscularly with blood from birds heavily infected with exo-erythrocytic parasites. All the donors had been treated with suppressive doses of drug from the time of inoculation, and the inocula were rich in uninucleate parasites. The presence of exo-erythrocytic forms in the brain and internal organs of the donors was verified by post-mortem examination made immediately after the blood had been withdrawn. In each experiment of the series all the chicks were inoculated with equal amounts of blood from the same source, the first dose of drug being given immediately after inoculation. The dose of drug varied in the different experiments, but the lowest dosage was sufficient to suppress the development of parasites in the erythrocytes (Table 2). The drugs were given once daily and dosing was continued until the 28th day if death had not occurred previously.

In the forty-seven birds treated with drugs the average day of appearance of parasites (uninucleate) in the blood was the 9th as compared with the 5th in the twenty-seven untreated control birds. The greater proportion of the drugged birds died, the

Table 2. *The effect of drug treatment upon infections of* Plasmodium gallinaceum *induced by inocula from donors heavily infected with exo-erythrocytic parasites*

Drug	Dose	Average day of appearance of parasites in blood	No. of chicks surviving	Average day of death	No. of infections sterilized
Pamaquin	0·64	10 (6)	2 (6)	13·5 (4)	0 (6)
Pamaquin	0·32	8·7 (10)	2 (10)	13 (8)	0 (10)
Pamaquin	0·08	10·5 (2)	2 (2)	—	0 (2)
Mepacrine	5·12	9 (19)	0 (19)	12 (19)	0 (19)
Mepacrine	0·64	9·5 (2)	0 (2)	11 (2)	0 (2)
Quinine	10·24	9 (8)	1 (8)	13 (7)	0 (8)
Average for 47 birds	—	9·4	7 (47) (15 %)	12·5	—
Controls	—	5 (27)	1 (27)	12 (26)	—

The dose of drug is calculated in mg./20 g. of body weight.
The number of chicks in each experiment is given in parentheses.

cause of death being the occlusion of the capillaries of the brain with exo-erythrocytic parasites. No bird was sterilized of its infection by the action of the drug, but in seven of the forty-seven drugged birds (15%), although uninucleate parasites appeared in the peripheral blood for a number of days which varied with different birds, indicating that exo-erythrocytic parasites were present in the internal organs, they disappeared later and the birds survived until the 28th day or longer.

Only one of the untreated control birds survived, but the greater number died from an intense erythrocytic infection before exo-erythrocytic parasites had become numerous.

In a further series of experiments (Table 3) eighty-nine chicks were inoculated with a strain of parasite which had been passaged intravenously at intervals of 24 hr. through normal chicks, the last passage having been performed 24 hr. previously. If the original inoculum is heavily infected with parasites a steady infection can be kept going, in small chicks, by daily intravenous passage of 0·5–1·0 c.c. of heparinized blood. Prolonged search of preparations of the brain and internal organs of these birds 24 hr. after the introduction of parasites failed to reveal any exo-erythrocytic parasites. As in the previous experiments, all the birds of a series were inoculated intramuscularly with equal quantities of parasitized blood diluted in citrate-saline solution, and taken from a single donor bird. The drugs were administered daily, from the day of inoculation, in doses similar to those of the previous experiment. Dosing was continued until the 28th day if death had not occurred previously. The average day upon which parasites (uninucleate) first appeared in the erythrocytes of the peripheral blood was ascertained for those birds in which the infection became patent on or before the 28th day; it was found to be 12 days later than in the previous experiment in which the inocula were taken from donors heavily infected with exo-erythrocytic parasites.

If birds failed to show an infection before the 28th day, they were classed as 'Survived' for purposes of comparison with the previous series of experiments, but they were studied further in order to discover whether they had been sterilized of their infection by the action of the drugs. In determining whether an infection had been eradicated, blood films were examined daily for a week following the termination of drug treatment on the 28th day. If no parasites were found, subinoculation tests were performed to prove whether the bird carried an infection of so low a grade that it had not been detected in blood films. 0·7–1·0 c.c. of heparinized blood from the chick under investigation was injected intravenously into each of two normal chicks and blood films were examined daily from the 5th–18th day following inoculation. If the chicks remained free from infection during this period, the donor was considered to have been sterilized of its infection.

Table 3. *The effect of drug treatment upon infections of* Plasmodium gallinaceum *induced by inocula from donors in which no exo-erythrocytic parasites were found*

Drug	Dose	Average day of appearance of parasites in blood	No. of chicks surviving	Average day of death*	No. of infections sterilized
Pamaquin	0·64	26 (11)	7 (13)	22 (6)	2 (13)
Pamaquin	0·32	26 (10)	10 (13)	23 (3)	3 (13)
Pamaquin	0·08	17·5 (2)	0 (2)	20 (2)	0 (2)
Mepacrine	5·12	24·8 (19)	10 (23)	22·5 (13)	4 (23)
Mepacrine	0·64	16·5 (2)	0 (2)	24 (2)	0 (2)
Quinine	10·24	21 (11)	5 (13)	22 (8)	2 (13)
Average for 66 birds	—	22	32 (66) (48%)	22	11 (66)
Controls	—	5 (23)	0 (23)	11 (23)	—

* This figure is calculated from the deaths which occurred on or before the 28th day.
The dose of drug is calculated in mg./20 g. of body weight.
The number of chicks is given in parentheses.
Birds which lived for more than 28 days are classed as 'surviving'.

From a comparison of the two sets of experiments (Tables 2 and 3) it will be seen that the proportion of drugged birds which survived until the 28th day was greater (32/66) in the series inoculated from donors in which no exo-erythrocytic parasites could be found than in those (7/47) inoculated from donors in which such forms were numerous. Whereas none of the birds inoculated with parasites from donors heavily infected with exo-erythrocytic parasites was sterilized of infection by the drugs, eleven out of sixty-six of those which received inocula from birds in which no exo-erythrocytic forms had been found were sterilized of their infections.

In order to discover whether the duration of drug

treatment had an effect upon the proportion of infections sterilized, groups of chicks inoculated with strains of *P. gallinaceum* which had been passaged, in one case ten times, and in the other case nineteen times, at 24-hourly intervals through young chicks, were treated with mepacrine, pamaquin, or quinine for 6 days only. The chicks were inoculated intramuscularly with equal amounts of citrated blood, and treated with drugs in similar doses to those used in previous experiments, the first dose being given on the day of inoculation (Table 4). In five out of 13 birds the infections were eradicated. In those birds in which the infections were not sterilized the incubation period was longer than in the untreated controls, but shorter than when treatment was prolonged (Table 3). When the infections became patent parasites in all stages of development were seen in the peripheral blood.

Table 4. *The effect of drug treatment, given on the first six successive days following inoculation, upon a strain of* Plasmodium gallinaceum *which had been passaged ten or nineteen times through chicks at 24-hourly intervals*

Drug	Dose	Average day of appearance of parasites in blood	No. of infections sterilized
Pamaquin	6 × 0·32	14 (3)	2 (5)
Mepacrine	6 × 5·12	23 (3)	1 (4)
Quinine	6 × 10·24	14 (2)	2 (4)
Controls	—	5 (6)	—

The dose of drug is calculated in mg./20 g. of body weight.

The number of chicks is given in parentheses.

The sterilization of infections of *P. gallinaceum* in young chicks with pamaquin, mepacrine, or quinine is not, therefore, dependent upon prolonged treatment, but can be effected by short courses of treatment. The factor which determines whether sterilization of infection with these drugs is possible appears to be the nature of the inoculum. Only those infections which have been derived from donors in which no exo-erythrocytic parasites could be found at the time when the blood for the inoculum was withdrawn were eradicated. The results of drug treatment, however, were variable, some birds of a series being sterilized of their infections whereas others were not, though the inocula were similar in amount and origin, and similar doses of drug were administered at similar intervals. Infections produced by strains of *P. gallinaceum* derived from hosts heavily infected with exo-erythrocytic parasites, resembled sporozoite-induced infections (Table 5) in their insensitivity to the drugs: in neither type of infection was sterilization obtained.

IV. A COMPARISON OF PROPHYLACTIC AND CURATIVE TREATMENT OF BLOOD-INOCULATED INFECTIONS OF *PLASMODIUM GALLINACEUM*

As eradication of infection of *P. gallinaceum* was not influenced by the duration of drug treatment when this was begun immediately after inoculation, the effect of delay in the administration of drugs was studied. A comparison was made of the effect of prophylactic and curative treatment in groups of birds which had received similar inocula intramuscularly. In prophylactic treatment a dose of drug was given immediately before inoculation, and thereafter once daily until the 28th day, if the birds survived for this period. In curative treatment the first dose was withheld until the 6th–7th day, when a heavy erythrocyte infection was established, after which daily doses were administered until death or the 28th day, whichever was the earlier.

Table 5. *The effect of drug treatment upon sporozoite-induced infections of* Plasmodium gallinaceum

Drug	Dose	Average day of appearance of parasites in blood	No. of chicks surviving	Average day of death*	No. of infections sterilized
Pamaquin	0·64	7 (6)	3 (6)	9 (3)	0 (6)
Pamaquin	0·32	7 (2)	0 (2)	11 (2)	0 (2)
Mepacrine	5·12	7 (8)	1 (8)	9 (7)	0 (8)
Quinine	10·24	6·5 (4)	0 (4)	7 (4)	0 (4)
Controls	—	6 (8)	0 (8)	7 (8)	—

* This figure is based upon the deaths which occurred on or before the 28th day. Birds which lived for more than 28 days are classed as 'surviving'.

The dose of drug is calculated in mg./20 g. of body weight.

The number of chicks is given in parentheses.

In each of the five experiments chicks of the same age group were inoculated intramuscularly with equal quantities of heavily infected citrated blood. The strain of parasite had been passaged previously through normal chicks at 24-hourly intervals for eight or more serial passages. Of the seventeen birds treated prophylactically (Table 6) ten were sterilized

of their infections by the drugs, whereas in none of the fourteen birds treated curatively were the infections eradicated.

Within 24 hr. of the commencement of curative treatment the parasites showed degenerative changes due to the action of the drugs, but, although within a few days mature parasites were no longer seen in the peripheral blood, uninucleates were found and became numerous before death. These uninucleate parasites, presumably, were formed from the exo-erythrocytic forms whose development was unimpeded by the action of the drugs. All the birds treated curatively died between the 18th and 22nd day, and post-mortem examination revealed heavy infections of exo-erythrocytic parasites in the brain. The birds in which prophylactic treatment failed to achieve a radical cure survived until the 28th day or longer.

Exp. 1. In the first experimental series chicks were inoculated intramuscularly with equal quantities (0·5 c.c.) of citrated blood from a bird heavily infected with both erythrocytic and exo-erythrocytic parasites. Eight of the birds were treated daily, from the day of inoculation, with suppressive doses of pamaquin (0·32 mg./20 g.), quinine (20·48 mg./20 g.), or mepacrine (5·12 mg./20 g.), and eight remained untreated as controls. Heavy erythrocytic infections developed in all the untreated control birds and death occurred between the 8th and 14th days. Exo-erythrocytic parasites were found in the brain or internal organs of all the birds, except one which died on the 8th day. In the eight drugged birds the average time of survival (13·5 days) was longer than in the controls (11 days). Exo-erythrocytic parasites were found in all the drugged birds

Table 6. *A comparison of the results of prophylactic and curative treatment upon blood-inoculated infections of* Plasmodium gallinaceum *from donors in which no exo-erythrocytic parasites were found*

No. of exp.	Drug	Dose	Average day of appearance of parasites in blood	Average day of death	No. of infections sterilized
1	Mepacrine	5·12 P.	32 (1)	—	2 (3)
	Mepacrine	5·12 C.	5 (4)	22	0 (4)
2	Mepacrine	5·12 P.	27 (2)	29	1 (3)
	Mepacrine	5·12 C.	5 (3)	18	0 (3)
3	Quinine	20 P.	22 (2)	—	2 (4)
	Quinine	20 C.	5 (2)	19	0 (2)
4	Pamaquin	0·32 P.	39 (1)	—	2 (3)
	Pamaquin	0·32 C.	5 (2)	19	0 (2)
5	Pamaquin	0·32 P.	28 (1)	—	3 (4)
	Pamaquin	0·32 C.	5 (3)	18	0 (3)

The dose of drug is calculated in mg./20 g. of body weight.
The number of birds is given in parentheses.
P. = prophylactic; C. = curative.

As in each experiment both the birds treated curatively and those treated prophylactically were inoculated with parasites from a similar source, the difference in the course of infection must be due to the difference in timing of the drug treatment. Delay in the administration of mepacrine, quinine or pamaquin appeared to favour the development of exo-erythrocytic parasites. This deduction is opposed to that of Adler & Tchernomoretz (1941) who found that when quinine was given daily, in suppressive doses (150 mg./kg.), to fowls inoculated with emulsions of organs containing exo-erythrocytic stages of *P. gallinaceum*, the drug not only eliminated erythrocytic development, but stimulated the multiplication of exo-erythrocytic forms. As this conception raises a point of fundamental importance in the chemotherapy of avian malaria, the following experiments were made in order to obtain more data on the problem.

but the infections were, on the whole, lighter than in the controls.

Exp. 2. A second, similar experiment was performed with inocula taken from a bird in which, although the blood infection was heavy, few exo-erythrocytic parasites were present at the time when the blood was withdrawn. As in the previous experiment the erythrocytic infection in the controls became patent on the 5th day, but the survival time was longer. Two birds died on the 12th day, but no exo-erythrocytic parasites were found. In the three birds which died between the 15th and 19th days exo-erythrocytic parasites were numerous. Three birds which survived until the 28th day were killed, but post-mortem examination failed to reveal any exo-erythrocytic parasites. Of the birds under drug treatment four survived for more than 28 days. In three of these birds uninucleate parasites were found in the erythrocytes for varying periods during

treatment, indicating that exo-erythrocytic parasites were present in the internal organs, though in numbers too small to be lethal. Although no uninucleate parasites were seen in the erythrocytes of the 4th bird during the 28-day treatment, a low-grade infection of normal parasites developed in the blood from the 35th to 41st day. The remaining four birds receiving drug treatment died between the 13th and 24th days, and all proved to have exo-erythrocytic infections of varying intensity.

Exp. 3. A third series of birds was inoculated intramuscularly with a dilution of citrated blood from a donor in which no exo-erythrocytic parasites were found at the time when the blood was withdrawn. Of eleven control birds five died on or before the 17th day from heavy infections of the blood; exo-erythrocytic parasites were found in two only and were not numerous. Exo-erythrocytic forms were found, chiefly in the brain, in all four birds which died between the 18th and 28th days. Two birds survived the acute infection and were killed on the 28th day, but no exo-erythrocytic parasites were found in the internal organs. Of eleven birds treated with drugs, only one died during the 28-day course of treatment: smears of the brain revealed the presence of exo-erythrocytic parasites. In two of the remaining drugged birds uninucleate parasites were seen in the erythrocytes, in one case for 2 days and in the other for 5 days, during drug treatment, but no exo-erythrocytic parasites were found at post-mortem examination made on the 28th day. No parasites were seen in the erythrocytes of the remaining eight birds during drug treatment, and no exo-erythrocytic parasites were found at post-mortem examination made on the 28th day.

In all three experiments many of the control birds died from erythrocytic infections which reached lethal intensity before exo-erythrocytic parasites had become numerous. In the birds undergoing drug treatment the erythrocytic infection was suppressed, and death therefore was delayed until the exo-erythrocytic parasites had had time to develop in the capillaries of the brain. In none of the three experiments is there any evidence, however, that the exo-erythrocytic infection was heavier in the drug-treated birds than in the controls. In Exp. 3 only one drugged bird died during the period of treatment, and the number of exo-erythrocytic parasites observed was not large, whereas they were found in six controls, in three of which they were numerous,

No evidence was obtained that the drugs in suppressing the development of erythrocytic parasites produced an intensification in the development of the exo-erythrocytic forms. In some birds undergoing drug treatment it was observed that though exo-erythrocytic parasites became sufficiently numerous to produce a patent infection of uninucleate parasites in the peripheral blood their development was halted and their numbers diminished, as shown by the gradual decrease in numbers and disappearance of uninucleate parasites. Thus, in spite of the suppression of the erythrocytic cycle of development by the action of the drugs, well-established infections of exo-erythrocytic parasites failed to continue their development.

The disappearance of uninucleate parasites from the peripheral blood was accompanied by the appearance of large numbers of young erythrocytes, as occurs at the crisis of a normal, uninhibited blood infection. Birds in which such inhibited infections had passed into a chronic state were found to be resistant to superinfection by intravenous injection with massive doses of erythrocytic parasites. Blood infections of normal intensity are thus not essential to the development of full immunity. These observations are in agreement with those of Lourie (1934), who found that in *P. cathemerium* immunity to superinfection was as strong in birds in which the acute stage of infection had been completely suppressed by quinine as in normal birds.

V. DISCUSSION

James (1939) showed that whereas in sporozoite-infected chicks exo-erythrocytic parasites were found in all birds examined within the first few days of the attack, in blood-inoculated infections they were seldom seen before the 10th day. He believed that the appearance of exo-erythrocytic parasites in infections induced by blood-inoculation might be due, in some instances, to the presence of one or more exo-erythrocytic parasites in the inoculum. Such forms are not infrequent in films made from the peripheral blood and heart blood of birds heavily infected with exo-erythrocytic schizonts and in a moribund condition. If these itinerant exo-erythrocytic parasites are introduced into a normal bird, it is to be presumed that they penetrate into the internal organs of the host and continue their development there.

Zain (1941) found that blood from fowls heavily infected with exo-erythrocytic schizonts, when inoculated into normal birds, produced infections characterized by the early appearance of exo-erythrocytic parasites in the brain. The time of appearance of exo-erythrocytic parasites depended upon the presence or absence of these forms in the donor. Birds inoculated with blood from donors free from exo-erythrocytic forms did not develop such forms until a later stage in the infection. He also attributes the early development of exo-erythrocytic schizonts in blood-inoculated infections to the presence of exo-erythrocytic parasites in the blood.

It was observed by Hawking (1945) that when exo-erythrocytic parasites, grown in tissue culture,

were inoculated into chicks the development of exo-erythrocytic parasites was very rapid, and the course of the infection resembled that produced by sporozoites rather than one resulting from trophozoite-inoculation. The presence of exo-erythrocytic parasites in the inocula would explain the rapid development of the exo-erythrocytic infection, and the early appearance of uninucleate parasites in drugged birds inoculated with blood from birds heavily infected with exo-erythrocytic forms. When infected blood from a host heavily infected with exo-erythrocytic parasites is passaged for 24 hr. through a normal chick, any exo-erythrocytic parasites present in the blood would have an opportunity of penetrating into the cells of the internal organs and thus be filtered out from the circulating blood. As, in the above experiments, not all the birds inoculated with blood which had been passaged one or more times, at 24-hourly intervals, through normal birds, were sterilized of their infections by the drugs, it is to be concluded either that not all the circulating exo-erythrocytic parasites had migrated into the tissues of the host, or that fresh exo-erythrocytic forms had developed from the erythrocytic parasites introduced.

As exo-erythrocytic parasites occurred at an early stage in only a small proportion of the blood-inoculated infections examined by him, James was of the opinion that the presence of exo-erythrocytic parasites in the circulating blood was fortuitous, and that they were introduced into only a small proportion of the birds inoculated with infected blood. In order to account for the appearance of exo-erythrocytic parasites in the third week in the majority of birds inoculated with infected blood, he believed that it was necessary to postulate that exo-erythrocytic forms could arise from erythrocytic parasites. That such a conversion could take place in *P. circumflexum* was shown by Coulston & Manwell (1941). Exo-erythrocytic forms developed in canaries inoculated with single erythrocytic parasites but not until the erythrocytic infections, which in the initial passages were light, became heavy as a result of massive infections in later passages. They suggested that the formation of exo-erythrocytic parasites occurs only when the defence mechanism of the host is severely taxed by a heavy infective dose of erythrocytic parasites. More recently, however, Downs (1947) found that heavy infections of exo-erythrocytic parasites occurred in the first sub-passage in chicks inoculated with a strain of *P. gallinaceum* derived from single erythrocytic parasites.

The results of experiments comparing the effect of prophylactic and curative treatments (Table 6), in which prophylactic treatment was found to sterilize a number of birds of their infections whereas curative treatment in every case failed, can best be explained by the conversion of erythrocytic into exo-erythrocytic forms. As, in each experiment, the groups of birds treated prophylactically or curatively were inoculated with equal quantities of blood from the same source, the fact that ten out of seventeen birds treated prophylactically, and none of the birds treated curatively, were sterilized appears to be significant. There are three possible explanations of these results. First, exo-erythrocytic schizonts may have been present in the blood inoculated into all the chicks, but early treatment with mepacrine, quinine or pamaquin eradicated them in most birds, whereas delay in treatment allowed them to multiply and become so numerous that the drug failed to check their development. Kikuth & Mudrow (1939) believed that plasmoquine (pamaquin) had an inhibitory effect upon the development of the exo-erythrocytic stages of *P. cathemerium*, though it did not eradicate them. Davey (1946) found that this compound, if given in doses so large that they produced toxic symptoms in the chicks, had an inhibitory effect upon exo-erythrocytic parasites of *P. gallinaceum*. He also confirmed Kikuth & Mudrow's observations upon *P. cathemerium*. Tonkin (1946), however, failed to observe any plasmodicidal effect of pamaquin upon exo-erythrocytic forms of *P. gallinaceum* grown in tissue culture when the drug was used in concentrations tolerated by the macrophages. The observations of James & Tate (1937*a*, *b*), Brumpt *et al.* (1937), Davey (1946), and other workers, all indicate that quinine and mepacrine have no antiplasmodial action upon the exo-erythrocytic stages of *P. gallinaceum* in chicks. When studying the effect of these drugs upon exo-erythrocytic stages of *P. gallinaceum* in tissue culture, Tonkin found that as quinine was toxic to the macrophages it was impossible to determine whether it had any direct effect upon the parasites, but mepacrine had none. There is no evidence, therefore, that birds inoculated with exo-erythrocytic parasites can be sterilized of their infection by mepacrine or quinine, and the inhibitory effect of pamaquin observed by some workers is insufficient to bring about radical cure. It is, therefore, improbable that exo-erythrocytic parasites were introduced into those birds which were sterilized of their infections.

The second possibility is that birds which were sterilized of their infection received no exo-erythrocytic parasites in the inoculum, whereas those in which an infection developed were inoculated with exo-erythrocytic parasites in addition to erythrocytic forms. As the birds in each series were inoculated at random from a common pool of infected blood, which was shaken between each inoculation, and as in all four experiments sterilization of infection was obtained only in those birds treated prophylactically, it is improbable that the inocula introduced into these birds were free from exo-erythrocytic parasites, whereas the inocula introduced into

birds which were not sterilized of their infections contained exo-erythrocytic parasites. Failure in obtaining sterilization of infection cannot, therefore, be attributed to the chance introduction of exo-erythrocytic parasites in the inoculum.

The third possibility is that only erythrocytic forms were present in the inocula, and that, whereas in the majority of birds treated prophylactically they were destroyed before they had penetrated into the cells of the internal organs and become converted into exo-erythrocytic forms, in the birds treated curatively the delay in administration of drug allowed this to take place. It must be assumed that in those birds treated prophylactically, but not sterilized of their infections, a few exo-erythrocytic parasites developed from erythrocytic forms before the drugs had time to exert their full effect.

In an attempt to obtain evidence of the conversion of erythrocytic into exo-erythrocytic parasites, extensive examinations were made of the internal organs of chicks injected intravenously with erythrocytic parasites of *P. gallinaceum*. Inocula of up to 1·0 c.c. of heparinized blood, heavily infected with a strain of *P. gallinaceum* which had been passaged through normal chicks at intervals of 24 hr., were used. Some of the birds were treated with suppressive doses of mepacrine, quinine or pamaquin, whereas others received no drug. No exo-erythrocytic parasites were found in the liver, spleen, brain or bone-marrow of birds killed 24–48 hr. after infection. In one bird treated with daily doses of 5·12 mg./20 g. of mepacrine and killed on the 4th day following inoculation, extensive phagocytosis of normal and infected erythrocytes was observed in the liver and spleen, but no exo-erythrocytic parasites were found. In a second bird, also examined on the 4th day and undergoing similar drug treatment, phagocytosis of normal and infected erythrocytes was observed in the liver and spleen, and a few exo-erythrocytic parasites were found in the spleen. Only one of these parasites had more than four nuclei. A few exo-erythrocytic parasites were, however, found on the 4th day in the spleen of birds not receiving any drug. Although in drug-treated birds phagocytosis of corpuscles containing parasites damaged by the drug was frequently seen, there was no indication that the formation of exo-erythrocytic parasites was more frequent in such birds than in those receiving no drug.

It had formerly been considered that, unlike *P. relictum* in canaries, blood-inoculated infections of *P. gallinaceum* could not be eradicated by such drugs as quinine and mepacrine, which are without effect upon exo-erythrocytic parasites. Although the blood infection could be suppressed by these drugs death resulted at a later stage from an exo-erythrocytic infection the development of which had been unaffected by the drugs. Sterilization of blood-inoculated infections of *P. gallinaceum* appeared, therefore, only to be possible with drugs which would eradicate completely the exo-erythrocytic parasites. It will be seen, however, from the experiments described above, that although eradication of such infections cannot be obtained in every case, a proportion of blood-inoculated infections of *P. gallinaceum* can be eradicated by mepacrine, quinine or pamaquin provided that exo-erythrocytic parasites either free in the inoculum or in the circulating white cells are not introduced in the inoculum. The failure to eradicate all such infections is probably due to the conversion of erythrocytic parasites into exo-erythrocytic forms.

SUMMARY

1. The development of exo-erythrocytic parasites of *Plasmodium gallinaceum* is more rapid and intense in chicks inoculated with blood from birds heavily infected with exo-erythrocytic parasites than in those inoculated with blood from birds in which no exo-erythrocytic parasites can be found.

2. If parasitized blood from a bird heavily infected with exo-erythrocytic parasites is inoculated intravenously into a normal bird and withdrawn 24 hr. later, it produces infections in normal birds characterized by a slow development of exo-erythrocytic parasites.

3. The proportion of birds which survived after inoculation with *P. gallinaceum* and daily treatment with suppressive doses of quinine, mepacrine or pamaquin, was greater when the inoculum was derived from donors in which no exo-erythrocytic parasites could be found (48 %) than in those inoculated with blood from donors in which exo-erythrocytic parasites were numerous (15 %). No birds inoculated with parasitized blood from donors heavily infected with exo-erythrocytic parasites were sterilized of their infections by the action of the drugs, whereas in eleven out of sixty-six of the birds inoculated with parasitized blood from birds in which no exo-erythrocytic parasites could be found the infections were eradicated by the drugs.

4. Blood-inoculated infections of *P. gallinaceum* may in some cases be eradicated by short courses of treatment with mepacrine, quinine or pamaquin.

5. Whereas blood-inoculated infections of *P. gallinaceum* derived from donors in which no exo-erythrocytic parasites could be found were sometimes eradicated by prophylactic treatment with quinine, mepacrine or pamaquin, it was not possible to eradicate the infections by curative treatment with similar doses of these drugs.

6. The difference in type of infection produced by parasitized blood from birds in which no exo-erythrocytic parasites could be found at the time of withdrawal of the blood and those in which

exo-erythrocytic parasites were plentiful appears to be due to the presence of exo-erythrocytic parasites in the circulating blood of the latter type of donor.

7. Sterilization by the action of quinine, mepacrine or pamaquin of blood-inoculated infections from donors in which no exo-erythrocytic forms could be found, appears to depend upon whether the erythrocytic parasites are killed before they can become converted into exo-erythrocytic forms.

8. No evidence has been obtained of the intensification of development of exo-erythrocytic parasites by the suppression of erythrocytic parasites with drugs.

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THE STABILITY OF PALUDRINE-RESISTANCE IN *PLASMODIUM GALLINACEUM* IN THE ABSENCE OF THE DRUG

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INTRODUCTION

It has recently been found possible to obtain strains of *Plasmodium gallinaceum* resistant to paludrine by treatment with the drug in gradually increasing doses (Bishop & Birkett, 1947, 1948; Williamson, Bertram & Lourie, 1947; Williamson & Lourie, 1947). This acquired resistance was proved by Bishop & Birkett to be retained over a period of 3 months, during which five cyclical passages through mosquitoes and chicks were made without intervening drug treatment. Thus paludrine-resistance was found to persist after changes in the environment of the parasite from the erythrocytes of the chick host to the tissue cells of the mosquito, and also after the parasite had undergone sexual development in the mosquito. These alternating passages through chicks and mosquitoes were associated, however, with phases differing in the rapidity of multiplication of the parasites and included a period when, in the form of sporozoites, they underwent no multiplication.

In studying the stability of acquired resistance to drugs it is important to observe not only the effect of sexual development in the resistant parasite, and of change of host, but also the effect of a prolonged period of rapid multiplication in the absence of the drug, when new protoplasm is constantly being synthesized and mutations may occur.

METHODS

The paludrine-resistant strain of *P. gallinaceum* described in a previous account as Strain B (Bishop & Birkett, 1948) has been used in the following experiments. This strain had been passaged intravenously at intervals of 48–72 hr. through chicks receiving oral doses of paludrine gradually increasing from 0·025 mg./20 g. of body weight once daily to 1·0 mg./20 g. twice daily.† The infected blood was mixed with a little heparin to prevent clotting. Volumes of the mixture varying from 0·1 to 0·5 c.c., according to the intensity of infection of the donor and size of the recipient, were injected into the jugular vein, the infective dose of parasites being sufficient to produce a heavy infection in the recipient on the 2nd or 3rd day.

The sensitivity of the paludrine-resistant strain of *P. gallinaceum* to the drug was compared with that of the normal parent strain by the method described previously (Bishop & Birkett, 1948). Briefly this method is as follows. A group of chickens was inoculated intravenously with approximately 50,000,000 parasites of the paludrine-resistant strain per bird. One half of the group was treated with paludrine twice daily for 3½ days and the other half received no drug and served as controls. A similar group of chicks was inoculated with 50,000,000 parasites of the normal parent strain, one-half being treated with a similar dosage of paludrine to that given to the birds infected with the paludrine-resistant strain, and the other half served as controls. The intensity of infection, assessed as the number of parasites per 500 erythrocytes, was determined by the examination of blood films made on the 4th day following inoculation.

EXPERIMENTAL

The degree of resistance acquired by the paludrine-resistant strain to 1·0 mg. doses of paludrine was first assessed after the strain had been passaged for 3 weeks through birds receiving this dose. The strain was found to produce light infections of healthy parasites in birds receiving 1·0 mg. of paludrine, a dose which inhibited the growth of the normal parent strain completely (Table 1). A further test

Table 1. *Comparison of intensity of infections produced by the normal strain of* Plasmodium gallinaceum *and a strain resistant to* 1·0 *mg. of paludrine*

The intensity of infection is expressed as the average number of parasites per 500 erythrocytes. The number of birds in each experiment is given in brackets.

		Dose of paludrine in mg./20 g.		
Strain	Control	1·0	0·8	0·6
Normal parent	400 (5)	0 (5)	—	—
Paludrine-resistant	265 (3)	36 (4)	—	—
Normal parent	1150 (6)	0 (6)	—	—
Paludrine-resistant	398 (5)	67 (5)	217 (5)	225 (6)

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† Throughout this account dosage is expressed in mg./20 g. of body weight of bird, and, unless stated otherwise, the drug was given twice daily.

made after the paludrine-resistant strain had been passaged for 9 weeks through birds receiving 1·0 mg. doses of paludrine gave similar results; light infections were produced in birds receiving 1·0 mg. doses of the drug, and heavier infections in birds receiving 0·8 and 0·6 mg. doses. In order that the untreated parent strain should be strictly comparable with the paludrine-resistant strain—with the exception that it had, at no time in its history, been in contact with paludrine—it, also, was passaged intravenously at intervals of 2–3 days through normal birds. In both the paludrine-resistant strain and the normal parent strain, therefore, the infections were acute throughout the period of the experiments.

Having proved that the paludrine-resistant strain made after 1 year and 2 weeks free from drug. The degree of resistance exhibited by the strain during this period did not change materially. The intensity of infection produced by the paludrine-resistant strain in birds receiving 1·0 mg. doses of the drug varied, but, with the exception of one experiment, the mean intensity of infection in each group of birds was not less than that produced by the strain before it was passaged through drug-free birds (Table 1). With doses of 0·1 mg., which inhibited the growth of the normal parent strain, infections produced by the paludrine-resistant strain were frequently heavy.

The intensity of infection produced by the paludrine-resistant strain in birds of any one group receiving 0·1–1·0 mg. doses of the drug was more

Table 2. *Comparison of intensity of infections produced by the normal strain of* Plasmodium gallinaceum *and the paludrine-resistant strain after passage through untreated chicks*

The intensity of infection is expressed as the average number of parasites per 500 erythrocytes. The number of birds in each experiment is given in brackets.

Strain	No. of passages through untreated birds	Duration of drug-free period in weeks	Control	Dose of paludrine in mg./20 g. 1·0	0·9	0·8	0·6	0·5	0·1
Normal parent	—	—	1016 (4)	—	—	—	1 (2)	—	—
Paludrine-resistant	58	19	831 (3)	146 (4)	—	314 (1)	949 (4)	—	—
Normal parent	—	—	1020 (6)	—	—	1 (6)	—	—	—
Paludrine-resistant	63	22	642 (6)	168 (5)	78 (4)	104 (4)	—	—	—
Normal parent	—	—	804 (6)	—	—	—	2 (5)	—	—
Paludrine-resistant	67	25	837 (6)	20 (4)	—	211 (5)	58 (4)	—	—
Normal parent	—	—	948 (4)	—	—	—	—	—	1 (6)
Paludrine-resistant	136	52	980 (6)	608 (6)	—	—	—	1203 (6)	828 (6)
Normal parent	—	—	652 (4)	—	—	—	—	—	1 (5)
Paludrine-resistant	137	53	518 (6)	70 (6)	—	—	—	192 (4)	445 (6)
Normal parent	—	—	852 (4)	—	—	—	—	—	1 (6)
Paludrine-resistant	140	54	275 (6)	125 (6)	—	—	—	188 (6)	315 (5)

of *P. gallinaceum* was definitely resistant to doses of paludrine as great as 1·0 mg., a substrain was passaged intravenously through normal, untreated birds at intervals of 2–3 days. After 19 weeks, during which the paludrine-resistant strain was passaged 58 times through normal birds, its sensitivity to paludrine was compared with that of the normal parent strain (Table 2). The paludrine-resistant strain produced infections of healthy parasites in birds receiving 1·0, 0·8 and 0·6 mg. doses of the drug. In birds treated with 0·6 mg. doses of paludrine, a dose which inhibited the growth of the normal strain, the infections produced by the paludrine-resistant strain were very heavy.

The sensitivity of the paludrine-resistant strain to the drug was tested after further passages through birds receiving no drug (Table 2), the final test being variable than in the untreated control birds of that group, and also more variable than in the controls of the normal parent strain. This variability, however, was not greater in the final tests, made after the paludrine-resistant strain had been maintained for 1 year and 2 weeks in the absence of the drug, than after only 19 weeks.

DISCUSSION

The earliest observations upon the stability of acquired drug-resistance in the absence of the drug were made by Roehl, and Browning (1908), in Ehrlich's laboratory. Roehl (quoted by Ehrlich, 1907) passaged a fuchsin-fast strain of *Trypanosoma brucei* through normal untreated mice and found that resistance to the drug was retained after 36

passages. In another fuchsin-fast strain of trypanosomes Browning (quoted by Ehrlich, 1907) found that resistance to the drug persisted through 25 drug-free passages covering a period of 2 months, but was lost after 4½ months (40th passage). A trypan-blue resistant strain proved to be still resistant after 5 months, and an atoxyl-resistant strain after 9¾ months. Another atoxyl-resistant strain, which retained its resistance to this drug through 67 drug-free passages over a period of 6 months, had lost it by the 87th passage at the end of 7¾ months.

Since these early observations data have been accumulated upon the stability, or loss, of acquired resistance to drugs both in trypanosomes and in other organisms. Observations upon the stability of drug-resistance in trypanosomes by Murgatroyd & Yorke (1937) showed that, in strains of *T. rhodesiense* in mice, acquired resistance to a drug was retained indefinitely in the absence of the drug, if this were one which produced resistant strains rapidly. Thus an acriflavine-fast strain retained its resistance for 3 years, a tryparsamide-fast strain for 5½ years, and an atoxyl-fast strain for 7½ years in the absence of the drugs. Resistance to Bayer 205, which developed much more slowly than did resistance to acriflavine and the aromatic compounds of arsenic and antimony, was found to have been partially lost within 1 year, and to have disappeared when the strain was tested again 3 years later.

Robertson (1929) observed a gradual diminution in resistance to acriflavine when a culture of *Bodo caudatus*, which had acquired resistance to this drug, was cultivated continuously on drug-free media; no culture raised to any considerable degree of drug-resistance, however, lost this character completely during the period of the experiments, which did not exceed 1 year.

Resistance to sulphonamides and antibiotics in bacteria, acquired both by treatment *in vitro* and *in vivo*, has been studied recently by many workers and observations upon its stability are numerous, but only a few examples can be discussed here. Thus, Dettwiler & Schmidt (1940) found that in *Pneumococcus* Type III, rendered resistant to sulphapyridine by treatment during passage through mice, no appreciable loss in resistance occurred after twelve serial passages through untreated mice. Resistance had, however, diminished slightly by the 26th passage and had disappeared completely by the 53rd passage. As a result of later work with strains of pneumococci made highly resistant to sulphapyridine, Schmidt, Sesler & Dettwiler (1942) found that, in the case of one strain, passage through 223 untreated mice, and in the case of another strain, passage through 213 untreated mice, did not alter the degree of resistance to this drug. These workers conclude that resistance to sulphonamides, once firmly established, is retained almost indefinitely, though it may be lost if the initial resistance acquired is less complete.

A strain of *Mycobacterium raniae* made highly resistant to sulphathiazole was found by Yegian, Budd & Middlebrook (1946) to have preserved its resistance unchanged through 35 transfers in drug-free medium over a period of 10 months. Similar results were obtained by Oakberg & Luria (1947) with *Staphylococcus aureus*; acquired resistance to sulphathiazole was retained after 15 transfers in the absence of the drug.

According to Demerec (1945) strains of *Staph. aureus* resistant to penicillin were developed *in vitro* with great ease and retained their resistance after growth in drug-free media. Strains isolated after one transfer through penicillin media acquired a resistance which was stable. Opposed to these observations are those of Klimek, Cavallito & Bailey (1946), who found that whereas strains of *Staph. aureus* resistant to penicillin, streptomycin and other specified antibiotics could be developed in culture, reversal to the sensitive state was rapid regardless of the time required to develop the resistance and of the degree of resistance acquired.

Davies, Hinshelwood & Pryce (1945), in a study of the adaptation of *Bact. lactis aerogenes* to proflavine, divide the development of resistance to the drug into the following stages: (1) incomplete· (2) complete but unstable—i.e. partially lost when the organism is cultivated in a drug-free medium; (3) completely stable, a state reached only after prolonged treatment with the drug.

It can be concluded that whereas in some organisms partial or total loss of acquired resistance to drugs may occur as a result of growth in a drug-free environment, in others, if strongly developed, it appears to be stable over long periods. It must be stressed, however, that the duration of drug-resistance in different organisms is not comparable, as the generation time may vary greatly with different organisms and with the environmental conditions.

The strain of *Plasmodium gallinaceum* in which the stability of resistance to the drug has been studied had attained a high degree (forty-fold) of resistance before it was transferred to drug-free chicks. Resistance to the drug proved to be a stable character, persisting after passage of the strain through untreated chicks for more than 1 year. Throughout this period the infection was maintained in an acute state in which multiplication of the organisms was rapid. If non-resistant forms did arise during this period of rapid reproduction, either they must have been so rare that their numbers remained insignificant, or they were at a selective disadvantage to the drug-resistant forms since overgrowth of the latter did not occur. It would appear, therefore,

from these and earlier experiments (Bishop & Birkett, 1948), that paludrine-resistance in *P. gallinaceum* is stable, and persists both after long periods of rapid multiplication of the resistant strain in the absence of the drug, and after the sexual development of the parasite in the mosquito vector.

SUMMARY

Acquired resistance to paludrine in *Plasmodium gallinaceum* was retained undiminished after passage at frequent intervals through untreated chicks for more than 1 year.

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SULPHADIAZINE-RESISTANCE IN *PLASMODIUM GALLINACEUM* AND ITS RELATION TO OTHER ANTIMALARIAL COMPOUNDS

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1. INTRODUCTION

Acquired resistance to paludrine was first described, in avian malaria, by Bishop & Birkett (1947) and Williamson, Bertram & Lourie (1947), working independently. Attempts by Williamson and his co-workers to produce a strain of *Plasmodium gallinaceum* resistant to sulphadiazine failed, though treatment with the drug was continued for a period of 27 months. As already reported (Bishop & Birkett, 1948; Bishop & McConnachie, 1948) Bishop and her collaborators have obtained a strain of *P. gallinaceum* in which a thirty-two-fold increase in resistance to sulphadiazine has been induced as a result of constant treatment with the drug. The present account describes the methods used to induce resistance to sulphadiazine in strains of *P. gallinaceum* and the reaction of these strains to paludrine and other antimalarial drugs.

2. THE DEVELOPMENT OF RESISTANCE TO SULPHADIAZINE IN *PLASMODIUM GALLINACEUM* IN CHICKS

In developing a strain of *P. gallinaceum* resistant to paludrine, Bishop & Birkett (1948) used doses of the drug gradually increasing in size, beginning with a dose (0·025 mg./20 g. of body weight once daily†) so small that it did not suppress the blood infection completely, though it had, at first, an adverse effect upon some of the parasites. The dose of drug was gradually increased as the strain developed in resistance, until infections of normal intensity and appearance were produced in chicks receiving doses of 1·0 mg. Throughout the period of 'training' the increases in the dosage of drug were never so great as to inhibit the infection in the peripheral blood of the chicks, and the strain was maintained in a constantly patent state by intravenous injection of large inocula of infected blood, every second or third day, into chicks 45–65 g. in weight. The blood used for the inoculum was mixed with a little heparin in saline to prevent coagulation.

A similar method was used in inducing resistance to sulphadiazine. In the early stages of drug treatment a dose of 0·625 mg. of sulphadiazine was used which, when given to birds injected intravenously with blood heavily infected with *P. gallinaceum* (0·25-0·5 c.c.), did not inhibit the development of the infection in the peripheral blood very drastically though it had an adverse effect upon many of the parasites. In successive passages at intervals of 2–3 days through birds receiving this dose of drug, the proportion of abnormal to normal parasites gradually decreased until only parasites of normal appearance were seen. The dose was then increased to 1·25 mg. which, when given twice daily, inhibits the growth of the normal parent strain almost completely. Again large numbers of the parasites in all stages of degeneration were seen, the schizonts being affected much more rapidly than the gametocytes. During subsequent passages through chicks receiving 1·25 mg. of sulphadiazine the strain gradually

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† Unless stated otherwise drugs were given twice daily, the dosage being calculated in mg./20 g. of body weight of bird. The doses were given in aqueous solution, orally by catheter tube, into the stomach. Sulphadiazine, in powder form, was made up in a solution of gum acacia.

became adjusted to this dose of drug. The dose was gradually increased until infections of parasites normal in appearance were produced in chicks receiving 20 mg. twice daily. This relatively large dose had no toxic effect upon the chicks which gained in weight steadily during treatment. No attempt was made to increase the dose above this level.

The degree of drug resistance acquired (Table 1) was assessed from time to time by the method used previously with the paludrine-resistant strain (Bishop & Birkett, 1947, 1948). The intensity of infection produced by the intravenous injection of 50,000,000 parasites per chick was compared in (*a*) the resistant strain in groups of 3–6 chicks treated with doses of sulphadiazine twice daily for 3½ days, and (*b*) similar groups of chicks inoculated with the same number of parasites of the normal parent strain and receiving similar drug treatment. In both (*a*) and (*b*) groups of untreated birds served as controls. The intensity of infection was assessed, on the fourth day following inoculation, as the number of parasites per 500 corpuscles. In order that the normal parent strain should be strictly comparable, apart from drug treatment, with the sulphadiazine-resistant strain, it was maintained in a state of acute infection by intravenous passage through untreated chicks at intervals of 2–3 days.

Throughout the period of treatment with gradually increasing doses of sulphadiazine the intensity of infection of the resistant strain in treated birds was lower than that of the parent strain in untreated birds; this difference was still very marked when the resistant strain appeared, in all other respects, to be completely adapted to a particular concentration of drug. The results of tests made to assess the degree of resistance attained at different levels of drug treatment (Table 1) confirmed this. Not only were the infections produced by the sulphadiazine-resistant strain in drugged birds lighter than those of the normal parent strain in untreated birds, but those produced by the resistant strain in untreated birds were, in most cases, also lighter. Even when the resistant strain had been subjected to sulphadiazine for a period of the order of 2 years (Table 1, Exps. 6 and 7), and had passed a large part of the time in birds receiving 20 mg. doses of drug, infections produced both in normal and drugged birds were lighter than those of the normal parent strain in untreated birds, although the development of the resistant parasites appeared, in all other respects, to be normal. This low intensity of infection was not, therefore, a transitory effect due to imperfect adaptation to the drug; rather it suggests some relatively permanent change in the growth rate of the resistant strain.

Although sulphadiazine has been found to have an inhibitory effect upon the development of exo-erythrocytic forms of *P. gallinaceum* (Coggeshall,

Table 1. Plasmodium gallinaceum. *Development of resistance to sulphadiazine*

Exp.	Strain	Duration of S.D. treatment (in weeks)	No. of passages through treated birds	Total dosage of S.D. in g./20 g.	Max. dose during treatment in mg./20 g.	Control*	Dose of S.D. in mg./20 g. 20	5	2·5	1·25
1	Normal	—	—	—	—	806	—	—	5	—
	Resistant	26	38	0·344	2·5	578	—	—	145	—
2	Normal	—	—	—	—	476	—	—	1	—
	Resistant	44	66	0·822	2·5	198	—	—	37	—
3	Normal	—	—	—	—	1065	—	—	3	2
	Resistant	47	71	0·909	2·5	245	—	—	336	360
4	Normal	—	—	—	—	808	—	—	5	—
	Resistant	26	36	0·501	5	592	—	124	—	—
5	Normal	—	—	—	—	669	—	2	—	0
	Resistant	49	68	1·689	5	299	—	94	—	165
6	Normal	—	—	—	—	872	5	—	—	—
	Resistant	99	204	11·797	20	264	95	—	—	—
7	Normal	—	—	—	—	1161	—	—	—	1
	Resistant	111	224	14·177	20	280	51	—	—	—
				After one cyclical passage through *Aëdes aegypti*						
8	Normal	—	—	—	—	1074	—	—	—	3
	Resistant	53	71	1·674	5	445	—	147	—	401
9	Normal	—	—	—	—	581	—	—	—	1
	Resistant	60	80	1·977	5	1029	—	371	—	540

* The intensity of infection is expressed as the average number of parasites per 500 erythrocytes in groups of 3–6 birds. S.D. = sulphadiazine.

Porter & Laird, 1944; Tonkin, 1946), these forms may become numerous in birds infected with the sulphadiazine-resistant strain in spite of intensive treatment with the drug. Thus at the time of, or after, the crisis of the blood infection, birds receiving as much as 20 mg. of sulphadiazine twice daily may die from occlusion of the capillaries of the brain by exo-erythrocytic schizonts, and large numbers of these forms may be found in the liver and the spleen. Acquired resistance to the drug is not, therefore, limited to the phase of development in the erythrocytes, but extends to parasites undergoing development in other types of cell.

3. THE PERSISTENCE OF SULPHADIAZINE-RESISTANCE AFTER CYCLICAL PASSAGE THROUGH MOSQUITOES

Bishop & Birkett (1947, 1948) found that no loss in resistance to paludrine occurred when a strain of *P. gallinaceum* resistant to this drug was passaged five times through mosquitoes and chicks alternately without intervening drug treatment. The following experiments were made in order to discover whether resistance to sulphadiazine also was retained after cyclical passage through *Aëdes aegypti*. At the time when these experiments were made the sulphadiazine-resistant strain had been passaged through chicks treated with sulphadiazine in doses increasing to a maximum of 5 mg. twice daily. Except for the lower growth rate as compared with the normal strain, the resistant strain appeared to be unaffected by the drug.

Mosquitoes were fed upon birds infected with the sulphadiazine-resistant strain and in which the proportion of gametocytes was high. The mosquitoes were fed shortly after the bird had received a 5 mg. dose of the drug. On the 9th day following the blood meal the mosquitoes were dissected and heavy infections of sporozoites were found in the salivary glands. The sporozoites, suspended in half-strength Ringer solution, were inoculated intravenously into normal birds. When the infections became patent the birds were bled, the blood was inoculated into groups of chicks, and tests for resistance to 5 and 1·25 mg. doses of sulphadiazine were made by the method described above (p. 164). No loss in resistance was observed, the intensity of infections produced by the resistant strain after mosquito passage being equal to or greater than those produced by the strain before mosquito passage when treated with similar doses of drug (Table 1). In *Plasmodium gallinaceum*, therefore, acquired resistance to sulphadiazine, like resistance to paludrine, is unaffected by sexual development in the invertebrate host.

4. THE EFFECT OF SULPHATHIAZOLE, SULPHANILAMIDE AND SULPHAPYRIDINE UPON A STRAIN OF *PLASMODIUM GALLINACEUM* RESISTANT TO SULPHADIAZINE

It has been observed by many workers that in bacteria, organisms which have become resistant to one sulphonamide are also resistant to other members of the group, provided that all the compounds can be antagonized by *p*-aminobenzoic acid. Davies & Hinshelwood (1943) studied the development of resistance to sulphonamides in *Bact. lactis aerogenes* and found that in the early stages of training resistance is specific to the compound used, but later, when training is complete, resistance extends to other sulphonamides.

Sulphadiazine has the most potent action of all the sulphonamides upon *Plasmodium gallinaceum*. It is extremely well-tolerated by chicks and inhibits the development of the parasite when given in small doses. Thus 0·625–1·25 mg., when given twice daily for 3½ days, inhibits the blood infection almost completely. Its chemotherapeutic index, therefore, is relatively large. Sulphathiazole, sulphapyridine and sulphanilamide are less effective against the parasite and their toxicity to chicks is much greater; thus sulphanilamide produces serious loss in weight when given to chicks in doses of 8 mg. These compounds are, however, sufficiently active against *P. gallinaceum* to enable tests upon cross-resistance with sulphadiazine to be made.

The sulphadiazine-resistant strain used in the cross-resistance tests was resistant to doses of 20 mg. of the drug. This strain produced infections of normal parasites in birds treated with doses of sulphathiazole, sulphapyridine and sulphanilamide which inhibit the development of the untreated parent strain almost completely (Table 2). The intensity of the infections produced by the sulphadiazine-resistant strain in birds receiving these drugs was of the same order as that produced in birds treated with sulphadiazine (Table 1) and in the untreated controls (Table 2). In a strain in which resistance to sulphadiazine is well-established this resistance is not, therefore, specific, but extends to related compounds.

5. THE EFFECT OF MEPACRINE, PAMAQUIN AND QUININE UPON A SULPHADIAZINE-RESISTANT STRAIN OF *PLASMODIUM GALLINACEUM*

In bacteria it has been shown that acquired resistance to a drug may be specific to that compound or may extend to unrelated compounds. In attempting to

Table 2. Plasmodium gallinaceum. *The effect of sulphathiazole, sulphanilamide and sulphapyridine upon a strain resistant to* 20 *mg./*20 *g. sulphadiazine*

Compound	Strain	Control	Dose in mg./20 g. 10	5	2·5	1·25
Sulphathiazole*	Normal	759	4	10	—	—
	Resistant	187	227	198	—	—
Sulphathiazole*	Normal	891	2	—	36	—
	Resistant	105	103	—	—	—
Sulphathiazole*	Normal	1161	3	16	—	—
	Resistant	280	419	381	—	—
Sulphapyridine	Normal	665	4	39	—	—
	Resistant	336	146	272	—	—
Sulphapyridine	Normal	1161	4	105	—	—
	Resistant	280	275	331	—	—
Sulphanilamide	Normal	665	—	1	52	157
	Resistant	1023	5	43	270	—
Sulphanilamide	Normal	1161	4	1	—	—
	Resistant	280	201	187	—	—

* Thiazamide sodium (May and Baker), the soluble form of sulphathiazole, was used in these tests. The amount of drug administered gave the dosage of sulphathiazole stated. The intensity of infection is expressed as the average number of parasites per 500 erythrocytes in groups of 3–6 birds.

Table 3. Plasmodium gallinaceum. *The effect of mepacrine, pamaquin, quinine and* M 4430 *upon a sulphadiazine-resistant strain.*

Compound	Dose in mg./20 g.	Strain R	N	R	N	R	N
Control	—	264	872	196	1089	554	1178
Mepacrine	1 × 0·64	1	11	0	2	—	—
	1 × 0·32	—	—	141	819	—	—
Pamaquin	2 × 0·08	—	—	0	1	—	—
	2 × 0·04	2	536	0	6	9	39
	2 × 0·02	—	—	13	586	19	1116
	2 × 0·01	—	—	—	—	198	—
Quinine	2 × 0·8	9	18	—	—	5	8
	1 × 0·8	—	—	—	—	32	24
M 4430	2 × 0·4	267	1	—	—	628	42

R = sulphadiazine-resistant strain. N = normal parent strain.

The intensity of infection is expressed as the average number of parasites per 500 erythrocytes in groups of 3–6 birds. The doses of drug were given once (1 ×) or twice (2 ×) daily.

obtain some light upon the mode of action of antimalarial drugs and the method by which resistance to sulphadiazine is acquired in *P. gallinaceum*, it is of interest to discover whether resistance to sulphadiazine is specific to sulphonamides antagonized by *p*-aminobenzoic acid, or whether it extends to unrelated compounds of proved antimalarial action. The strain of *P. gallinaceum* resistant to 20 mg. doses of sulphadiazine, therefore, was tested for cross-resistance to mepacrine (atebrin), pamaquin (plasmochin) and quinine.

The minimum dose of mepacrine hydrochloride effective upon the normal parent strain of *P. gallinaceum* is 0·64 mg. once daily (Table 3). This dose, when given to chicks on four consecutive days, inhibited the growth of both the sulphadiazine-resistant strain and the normal parent strain. In birds treated with 0·32 mg. of mepacrine once daily for a similar period the growth of the resistant strain was poorer than that of the parent strain. This may have been due to the inherently lower growth rate of the resistant strain, as shown by the lighter infections

in untreated control birds as compared with the parent strain in untreated birds, and not to a greater sensitivity to mepacrine.

The minimum dose of pamaquin effective upon the normal parent strain when tested by the usual method is 0·08 mg. twice daily. With doses of 0·04 mg. twice daily growth was irregular, indicating that the drug had some slight effect upon the parasites; doses of 0·02 mg. twice daily were without effect. The development of the sulphadiazine-resistant strain was inhibited not only by 0·08 mg. of pamaquin (Table 3), but also by 0·04 and 0·02 mg. Most of the parasites which remained in the peripheral blood after the 3½-day course of treatment with these doses were abnormal in appearance and definitely affected by the drug. In developing resistance to sulphadiazine, therefore, *P. gallinaceum* has also developed a hypersensitivity to pamaquin.

The minimum dose of quinine dihydrochloride effective upon the normal parent strain of *P. gallinaceum* is 0·8 mg. when given once daily. In birds treated with these doses no significant difference in the growth of the sulphadiazine-resistant and normal parent strains could be observed (Table 3). The development of resistance to sulphadiazine does not, therefore, affect the sensitivity of *P. gallinaceum* to quinine.

Acquired resistance to high doses of sulphadiazine, therefore, though it confers resistance to related sulphonamides antagonized by *p*-aminobenzoic acid, does not confer resistance to pamaquin, mepacrine or quinine, even when administered in the minimum effective dose.

6. THE DEVELOPMENT OF RESISTANCE TO PALUDRINE (PROGUANIL) IN SULPHADIAZINE-RESISTANT STRAINS OF *PLASMODIUM GALLINACEUM*

Whereas resistance to sulphadiazine did not confer resistance to pamaquin, mepacrine or quinine it was found to confer resistance to paludrine (proguanil) (Bishop & McConnachie, 1948). The strain of *P. gallinaceum* resistant to 20 mg. of sulphadiazine, when tested with the minimum effective dose of paludrine (0·1 mg.), proved to be resistant to this dose. As Williamson *et al.* (1947) had found that their paludrine-resistant strain of *P. gallinaceum* showed no resistance to sulphadiazine, confirmation of our results by the study of other strains of *P. gallinaceum* resistant to sulphadiazine was necessary.

Two strains (A and B) of *P. gallinaceum* were developed from the normal parent strain of which subinoculations had been tested recently and found to have a normal sensitivity to the minimum effective doses of sulphadiazine (1·25 mg.) and paludrine (0·1 mg.) Strain A was maintained in a state of acute infection by intravenous injection of large inocula of heavily infected blood into chicks at intervals of 3–4 days. The chicks were treated with doses of sulphadiazine increasing gradually from 0·625 to 5 mg. as the strain developed in resistance to the drug. The drug was given twice daily with the exception of Sundays when no drug was given and Saturdays when only one dose was given. With strain B, 5 mg. doses were given from the beginning of the period of treatment. Whereas, during the 79-day period of treatment, strain A was maintained in a state of acute infection, strain B was passaged only 4 times and, as a result of the inhibitory effect of the 5 mg. doses of drug and the length of time between the passages, was in a latent state for the greater part of the time. The results of tests made after the 79-day period of treatment with sulphadiazine confirmed our earlier observations that resistance to sulphadiazine confers resistance also to paludrine (Table 4). Both strains produced infections in birds treated with 0·1 mg. of paludrine whereas the normal parent strain was inhibited by this dose of drug. Both strains also produced infections of healthy parasites in birds treated with 1·25 mg. doses of sulphadiazine, but in untreated birds and in birds receiving 5 mg. doses of the drug the growth of strain B was poorer than that of strain A, which suggested that strain B was, as yet, less well adapted to sulphadiazine than strain A. After treatment with 5 mg. doses of sulphadiazine twice daily for a further period of 21 days, during which strain A was passaged 6 times and strain B only once, the strains were tested again for resistance to both drugs (Table 4). Both strains produced good growths of parasites of normal appearance in birds receiving 0·1 and 0·5 mg. doses of paludrine, and the growth of strain B, both in untreated birds and in birds treated with 5 mg. of sulphadiazine, was much improved. After treatment with 5 mg. doses of sulphadiazine twice daily for a further 28 days, strain A was tested against twice daily doses of 1·0 mg. of paludrine, which is the largest dose of this drug which chicks will tolerate without loss of weight, and was found to be resistant to it.

Having proved that *P. gallinaceum*, when passaged for a long period through chicks under treatment with sulphadiazine, acquired resistance not only to sulphadiazine itself but also to paludrine, it was of interest to discover whether resistance to these compounds develops simultaneously or whether resistance to one compound precedes resistance to the other, and also to ascertain the minimum amount of sulphadiazine and the period of treatment required to produce resistance to paludrine. In an attempt to elucidate these problems a fourth strain of *P. gallinaceum*, strain C, was developed from the parent strain after this had, by subinoculation experiments, been proved to retain its normal sensitivity

Table 4. Plasmodium gallinaceum. *A comparison of the development of resistance to sulphadiazine and paludrine in strains treated with sulphadiazine and passaged through chicks at frequent (strain A) and infrequent (strain B) intervals*

Strain	Duration of S.D. treatment (in days)	No. of passages through treated birds	Total dosage of S.D. in mg./20 g.	Max. dose during treatment in mg./20 g.	Control	Dose of S.D. in mg./20 g. 5	1·25	Dose of paludrine in mg./20 g. 1·0	0·5	0·1
Normal	—	—	—	—	1026	—	2	—	—	0
Resistant A	79	22	137	5	145	80	147	—	—	173
Resistant B	79	4	315	5	44	10	98	—	—	142
Normal	—	—	—	—	617	—	1	—	—	0
Resistant A	100	27	302	5	232	67	184	—	191	285
Resistant B	100	5	430	5	541	139	347	—	611	585
Normal	—	—	—	—	793	2	—	—	—	0
Resistant A	128	34	532	5	113	50	—	71	—	116

The intensity of infection is expressed as the average number of parasites per 500 erythrocytes in groups of 3–6 birds.

Table 5. Plasmodium gallinaceum. *A comparison of the intensity of resistance to paludrine and sulphadiazine produced by treatment with sulphadiazine*

Strain	Duration of S.D. treatment (in days)	No. of passages through treated birds	Total dosage of S.D. in mg./20 g.	Max. dose during treatment in mg./20 g.	Control	Dose of S.D. in mg./20 g. 20	10	5	1·25	0·625	0·625*	Dose of paludrine in mg./20 g. 1·5	1·0	0·5	0·1
Normal	—	—	—	—	369	—	—	—	0	—	—	—	—	0	—
Resistant C	49	10	37	0·625	480	—	—	217	516	—	—	87	385	476	—
Normal	—	—	—	—	308	—	—	1	1	—	—	—	0	—	—
Resistant C	56	12	48	0·625	441	16	63	156	—	—	—	46	205	—	—
Normal	—	—	—	—	999	—	—	—	5	12	—	—	—	—	1
Resistant D	17	4	14	0·625	857	—	—	1	6	9	—	—	360	722	836
Normal	—	—	—	—	651	—	—	—	1	1	—	—	—	1	—
Resistant D	22	6	16	0·625	414	—	—	1	1	0	—	—	155	184	—
Normal	—	—	—	—	808	—	—	—	9	12	—	—	—	—	—
Resistant D	31	8	21	0·625	702	—	—	1	95	264	—	—	—	—	—
Normal	—	—	—	—	1110	—	—	—	3	—	203	—	—	—	1
Resistant E	10	3	7	0·625*	546	—	—	—	1	—	—	—	—	—	1
Normal	—	—	—	—	648	—	—	—	2	10	349	—	—	—	0
Resistant E	18	5	13	0·625*	241	—	—	—	0	—	71	—	—	—	0
Normal	—	—	—	—	888	—	—	—	3	3	—	—	—	—	1
Resistant EA	22	7	26	0·625†	536	—	—	—	194	408	—	34	206	—	834
Normal	—	—	—	—	513	—	—	—	9	21	—	—	—	—	1
Resistant E	38	9	23	0·625*	601	—	—	—	85	272	—	146	201	—	373
Normal	—	—	—	—	639	—	—	—	24‡	26	—	—	—	—	1
Strain F	3½	1	9	1·25	767	—	—	—	17	17	—	—	—	—	0

The intensity of infection is expressed as the average number of parasites per 500 erythrocytes in groups of 3–6 birds.

Unless indicated otherwise the drugs were given twice daily. Doses marked * indicate once daily dosage.

† 0·625 mg./20 g. once daily for 16 days followed by 0·625 mg./20 g. twice daily for 6 days.

‡ The high count was due to the high rate of infection in one bird which presumably failed to absorb the drug.

to the minimum effective doses of sulphadiazine (1·25 mg.) and paludrine (0·1 mg.). Strain C was passaged through chicks treated with doses of 0·625 mg. of sulphadiazine twice daily. This dose, when given over a period of 3½ days to chicks inoculated intravenously with 50,000,000 parasites, reduces the infection to a low level, though its effect is not very marked when the number of parasites introduced is very large as in the maintenance of a strain passaged at intervals of 2–4 days. After treatment with this dose for 49 days, during which it was passaged 10 times, strain C was proved to be resistant not only to doses of 0·5 and 1·0 mg. of paludrine, but also to 1·5 mg. (Table 5), a dose which when given several days consecutively produces a considerable loss in weight in many chicks. Strain C was tested after treatment with the same dosage of sulphadiazine for a further 7 days and again proved to be resistant to doses of paludrine up to 1·5 mg.; moreover, on both occasions, it was shown to be resistant to doses of sulphadiazine (1·25–20 mg.) much greater than that to which it had been exposed previously (0·625 mg.).

A fifth strain, strain D, was developed from the parent strain after its sensitivity to paludrine and sulphadiazine had again been proved to be normal. This strain also was passaged through chicks treated with 0·625 mg. of sulphadiazine twice daily, and, after a period of 17 days, during which a total of only 14 mg. of sulphadiazine was given, it was tested (Table 5) and found to have become resistant not only to the minimum effective dose of paludrine (0·1 mg.) but also to 1·0 mg., although it had as yet acquired no resistance to sulphadiazine. Five days later, after a total dosage of 16 mg. of sulphadiazine had been administered, the strain was tested again with the same result; it was found to be resistant to 1·0 mg. of paludrine, but its sensitivity to sulphadiazine was unchanged. After passage, for a further period of 9 days, through chicks treated with doses of 0·625 mg. of sulphadiazine twice daily, strain D was proved to have become resistant to low doses of sulphadiazine (1·25 mg.); later, after a further period of treatment with 0·625 mg. of the drug, slight resistance to 2·5 mg. of sulphadiazine developed.

As a high degree of resistance to paludrine had been induced in 17 days by passage through birds treated with a total of only 14 mg. of sulphadiazine, it was thought that a larger dose of the drug over a shorter period of time might also induce resistance to paludrine. Fifty million parasites of the normal parent strain were injected intravenously into a bird which was treated with 1·25 mg. of the drug twice daily for 3½ days after which the intensity of infection was only two parasites per 500 erythrocytes. 1·0 c.c. of blood was removed from the bird and injected intravenously into a normal untreated bird, and, 11 days later, when a heavy infection had developed, a test for resistance to paludrine was made but none had developed (strain F, Table 5). Thus a dosage of sulphadiazine which, when given over a period of 3½ days, is sufficient to inhibit the development of parasites, does not induce resistance to paludrine.

Three possible explanations of the failure to induce resistance to paludrine by treatment with 1·25 mg. of sulphadiazine for this short period present themselves: the total dosage of drug to which the strain had been submitted (9 mg.) was too small, the period of time during which the strain was exposed to the drug was too short, or the number (5×10^7) of parasites in the inoculum was not sufficiently large. If resistance to paludrine is induced by the selection of rare, resistant forms occurring in the normal population then the number of parasites present in the initial inoculum would be most important.

In order to test these possibilities two birds were inoculated intravenously, each with approximately 5×10^9 parasites of the normal parent strain. The birds were treated with 20 mg. of sulphadiazine twice daily on 4 successive days beginning with the day of inoculation. By the fourth day, after an aggregate dosage of 160 mg., the number of parasites in the peripheral blood was greatly reduced and the majority of those which remained appeared to be seriously affected by the drug. From each bird 0·25 c.c. of blood was inoculated intravenously into a normal bird. Within a period of 6 days a heavy infection of parasites normal in appearance developed in each recipient. After a further intravenous passage through normal birds each of these two strains was tested for resistance to 0·1 and 1·0 mg. of paludrine. No resistance was found. The treatment of the original two birds with 20 mg. of sulphadiazine was continued for a further period of 4 days, making a total dosage of 260 mg. of sulphadiazine over a period of 8 consecutive days. By the eighth day of treatment no parasites were observed in the blood of one bird and a few, normal in appearance, were seen in the blood of the other bird. These parasites had probably arisen from exo-erythrocytic forms (Bishop & Birkett, 1949). From each bird 0·25 c.c. of blood was passaged into a normal bird. When the infections in the blood of the recipients became heavy, the parasites were tested for resistance to 0·1 and 1·0 mg. of paludrine, but none was found. From these experiments it must be concluded that large doses of sulphadiazine given over short periods are not able to induce resistance to paludrine in *P. gallinaceum*, even when relatively enormous inocula are used.

At the time when strain D was first tested for resistance to paludrine the strain was already resistant to 1·0 mg. of the drug. The question therefore arose whether resistance to paludrine, when induced by treatment with sulphadiazine, develops

gradually or is immediately maximal. An attempt to solve this problem was made by studying the earliest stages in the development of resistance to paludrine as induced by sulphadiazine.

A new strain, E, was developed from the normal parent strain after this had again been tested for sensitivity to minimum doses of paludrine and sulphadiazine and found to be normal. It was passaged at intervals of 2–4 days through chicks receiving doses of 0·625 mg. of sulphadiazine once daily—a dose which has a slight effect upon the intensity of infection produced by the normal parent strain though few parasites are outwardly affected by it. After the strain had been subjected to this dose for 10 days it was found to have developed no resistance to paludrine. Treatment was continued with the same dose of drug for a further 8 days, but without effect upon the sensitivity of the strain towards paludrine (Table 5). A substrain (EA) of strain E was then passaged through chicks receiving 0·625 mg. of sulphadiazine twice daily for 5 days. This dose had an obvious effect upon the parasites and reduced the intensity of infection very appreciably. The infection resulting from this treatment was too low to make possible a test of the usual kind with inocula of 50,000,000 parasites, but it was possible to perform one with inocula of half this number per bird. With inocula of 25,000,000 parasites infections of normal parasites were produced in birds receiving 0·1, 1·0 and 1·5 mg. of paludrine, though the intensity of infection was slight. A further test, using inocula of 50,000,000 parasites per bird, was made upon strain EA (Table 5) after it had been subjected to twice-daily doses of 0·625 mg. of sulphadiazine for 6 days in addition to the previous treatment with the same dose once daily, making a total dosage of 26 mg. The strain was now found to be resistant to 0·1, 1·0 and 1·5 mg. doses of paludrine, though the infections produced in birds receiving 1·5 mg. doses were slight.

Strain E was tested a third time, after it had been treated with 0·625 mg. of sulphadiazine once daily for 38 days, and was found to have become resistant to 1·5 mg. doses of paludrine (Table 5). Both strain E and EA showed some resistance to sulphadiazine at the time when resistance to paludrine was demonstrated.

The minimum period of treatment with sulphadiazine with which resistance to paludrine was induced was 17 days with an aggregate dosage of 14 mg. of the drug. The dose used during this period (0·625 mg. twice daily) was definitely lethal to a large proportion of parasites of the parent strain. The same dose given once daily had a much less pronounced effect upon the parasites, and with it the period required to produce resistance to paludrine was more than double that required by the twice-daily dose.

From the foregoing experiments it can be concluded that, in *P. gallinaceum*, treatment with sulphadiazine induces a degree of resistance to paludrine which, even in its initial stage, extends over the full range of doses of paludrine tolerated by the chick, though the intensity of infection produced in birds receiving higher doses is slighter than in those receiving lower doses; resistance to paludrine develops more rapidly than resistance to sulphadiazine, and a high degree of resistance to paludrine may be attained before any increase in resistance to sulphadiazine can be demonstrated; finally, resistance to doses of sulphadiazine greater than those to which the strain had been exposed may develop as a result of prolonged treatment with the drug.

7. THE EFFECT OF M4430 UPON A SULPHADIAZINE-RESISTANT STRAIN OF *PLASMODIUM GALLINACEUM*

As strains of *P. gallinaceum* resistant to sulphadiazine have been shown to be resistant to paludrine, the effect of M4430 (N'-*p*-chlorophenyl-N^5-methyl-N^5-isopropylbiguanide) upon a sulphadiazine resistant strain was studied. A strain of *P. gallinaceum* resistant to paludrine had already been shown to be resistant to this methyl homologue (Bishop & Birkett, 1947, 1948; Williamson *et al.* 1947). It therefore seemed probable that, as sulphadiazine-resistant strains were resistant to paludrine they would likewise be resistant to M4430. This proved to be the case, for, when a sulphadiazine-resistant strain of *P. gallinaceum* was tested, in chicks, against the minimum dose of M4430 effective against the normal parent strain good growths of healthy parasites were obtained (Table 3).

8. THE EFFECT OF SULPHADIAZINE UPON PALUDRINE-RESISTANT STRAINS OF *PLASMODIUM GALLINACEUM*

It has been shown, in the experiments described above, that in infections of *P. gallinaceum* in chicks resistance to paludrine is rapidly produced by treatment with sulphadiazine. In order to discover whether the converse also is true the original paludrine-resistant strain of *P. gallinaceum* (Bishop & Birkett, 1947) was tested for resistance to sulphadiazine after the strain had been passaged, for over 18 months, at intervals of 2–3 days, through chicks treated with paludrine. Although in the early stages of the development of the strain the doses of paludrine had been small, during 15 months of this period 1·0 mg. was given twice daily, with the exception of Sundays when no drug was given and Saturdays when only one dose was given. As a result of

these tests good growths of parasites were obtained in chicks treated with 1·25 mg. of sulphadiazine for 3½ days, a dosage which inhibited the growth of the normal parent strain. The reaction of this strain to higher doses of sulphadiazine was not tested.

A second strain of *P. gallinaceum* was isolated from the normal parent strain in March 1948 and passaged at intervals of 2–3 days through chicks treated with doses of paludrine gradually increasing from 0·025 to 1·0 mg. twice daily. During the last 10 months of this period it has been passaged through chicks receiving 1·0 mg. of paludrine twice daily. It has been tested at frequent intervals for resistance to sulphadiazine, but up to the present (May 1949) its sensitivity to this drug has remained unchanged.

Williamson and his co-workers (Williamson *et al.* 1947; Williamson & Lourie, 1947) tested their paludrine-resistant strain of *P. gallinaceum* against sulphadiazine and found no cross-resistance. As pointed out in an earlier communication (Bishop & McConnachie, 1948) their strain had been subjected to paludrine for a much shorter period than had ours. Our results suggest that in strains of *P. gallinaceum* treated with paludrine resistance to sulphadiazine develops only after prolonged treatment with large doses of paludrine.

9. DISCUSSION

Whereas we have found that resistance to sulphadiazine can be induced with relative ease in *P. gallinaceum* by treatment of patent infections with the drug, Williamson and his colleagues (Williamson *et al.* 1947; Williamson & Lourie, 1947) failed to induce it in a strain which was treated with the drug for 27 months. A comparison of our methods with theirs shows certain differences. The age of the birds used in our experiments differed from those used by Williamson and his colleagues, ours being 1–2 weeks old and theirs 6–9 weeks. Whereas our strains were maintained by passage at intervals of 2–4 days, they passaged theirs only 44 times in 27 months. Twelve daily doses of sulphadiazine, each of 10 mg., were administered by Williamson and his co-workers during each passage, the treatment being begun on the first day that parasites were easily found in the peripheral blood. Our strains were maintained in a state of acute infection, parasites being numerous in the peripheral blood throughout treatment, whereas it can be assumed, from the methods which they employed and from the dose of drug administered throughout treatment, that, during part of each passage, their strains were not in a state of acute infection. Under such conditions the number of parasites in contact with the drug would be smaller than in a strain maintained in an acute state; moreover, parasites arising from exo-erythrocytic forms would be more frequent in the peripheral blood and in the inocula used at passage than in strains passaged every 2–4 days. It might be assumed that these differences in method were sufficient to determine the success or failure in inducing resistance to sulphadiazine if it were not that, in strain B, we induced resistance to this drug in 79 days, by a method very similar to that used by Williamson and his co-workers. Although the total daily dosage (10 mg.) was the same, in strain B the drug was given twice daily, and our chicks were younger than theirs. But these differences would appear to be too small to account for our success and their failure. It is possible that the facility for producing resistance to drugs differs between strains of *P. gallinaceum*.

Rollo, Williamson & Lourie (1948) have shown that resistance to paludrine does not develop if latent infections of *P. gallinaceum* are treated with that drug, even if treatment is continued for as long as a year. We ourselves are studying the effect of prolonged treatment with sulphadiazine upon latent infections of *P. gallinaceum*. In these experiments drug treatment is withheld until the acute infection has subsided and parasites are found only rarely in the peripheral blood. The results so far obtained show that strains resistant to sulphadiazine or paludrine are not produced from latent infections with the same facility as from acute infections. In one bird carrying a latent infection of *P. gallinaceum* an aggregate dose of 136 mg. of sulphadiazine, given over a period of 75 days, failed to produce resistance to sulphadiazine or to paludrine, whilst in a second bird similarly infected, 190 mg. of sulphadiazine, given over a period of 46 days, also failed to induce resistance to either drug. As parasites rarely appeared in the peripheral blood of these birds, it can be presumed that the main reservoir of infection was exo-erythrocytic parasites infecting the tissues of the internal organs. Failure to produce resistance to sulphadiazine or paludrine in latent infections may therefore be due to an innate insensitivity of the exo-erythrocytic parasites to the drug due to a different method of metabolism from that of erythrocytic forms; to their inaccessibility to the drug as a result of their locus in the cells of the internal organs; or to their slow rate of multiplication entailing, presumably, a correspondingly slow production of variants. The effect of more prolonged treatment with larger doses of sulphadiazine is being studied.

Our experiments upon *P. gallinaceum* in chicks have shown that resistance to paludrine is acquired rapidly by treatment of acute infections with sulphadiazine, and that once resistance to paludrine is acquired it extends to the full range of doses which the chick will tolerate. Bishop & Birkett (1947, 1948) found that resistance to the maximum dose of paludrine tolerated by the host developed in a strain of *P. gallinaceum* in chicks in 4½ months, as a result

of treatment of acute infections with gradually increasing doses of the drug. A high degree of resistance to paludrine was obtained in *P. gallinaceum*, in chicks, within about 3 months by Williamson *et al.* (1947), and in 15 weeks by Knoppers (1947). As the methods used by these workers differed somewhat one from another, the times taken to produce resistance are not strictly comparable. In developing their original paludrine-resistant strain Bishop & Birkett made no attempt to determine the minimum time required to establish resistance. It is therefore difficult, from these data, to compare the rate of development of resistance to paludrine as induced by paludrine or by sulphadiazine.

In order to obtain more comparable data we have recently treated a normal strain of *P. gallinaceum*, passaged at intervals of 2–4 days, with doses of paludrine gradually increasing from 0·025 mg. once daily, a dose which is practically devoid of effect upon infections of *P. gallinaceum* even when given over a long period, to 0·1 mg. twice daily, which is the minimum dose which inhibits the development of infection when given twice daily for 3½ days. The doses were increased as rapidly as possible without reducing the infections in the peripheral blood drastically. The method used in inducing resistance to this strain, therefore, was similar to that used in strain D, but whereas in strain D resistance to 1·0 mg. of paludrine twice daily was obtained after 17 days' treatment with minimum doses of sulphadiazine, in the new paludrine-treated strain resistance to 0·1 mg. of paludrine was obtained only after 70 days' treatment, and the strain was still sensitive to higher doses of the drug. Twenty-one days later, after further treatment twice daily with 0·1 mg. of paludrine and after an aggregate dosage of 8 mg. of drug, the strain proved to be fully resistant to 0·5 mg. of paludrine and slightly resistant to 0·8 mg.; but although parasites were found at the end of the 3½-day period of test in the blood of birds receiving 1·0 mg. of the drug twice daily, many of them were abnormal. Resistance to paludrine, therefore, develops more rapidly from treatment with sulphadiazine than from treatment with paludrine itself. Resistance to paludrine, as induced by sulphadiazine, is at once complete for all doses tolerated by the chick host, whereas resistance to paludrine, as induced by that drug, appears to develop by stages.

Recently, Adams & Seaton (1949) have induced resistance to paludrine in a West African strain of *P. falciparum* maintained by serial blood inoculation, by *in vivo* treatment with the drug for a period of 12 months. A strain of *P. vivax* has also been made resistant to this drug as a result of similar treatment (Lourie & Seaton, 1949). Schmidt, Genther, Fradken & Squires (1949) have obtained a two-thousand-fold increase in resistance to paludrine in *P. cynomolgi* in monkeys, and Hawking & Perry (1948) have also produced a high degree of resistance to this drug in the same species of malaria. Acquired resistance to paludrine is, therefore, not limited to *P. gallinaceum*, but can be produced experimentally in both monkey and human malaria. Whether resistance to paludrine can arise in human malaria under conditions of normal therapeutic treatment has not yet been demonstrated. It must be stressed that the conditions under which it has been induced experimentally are designed to favour its development.

Our experiments have shown that in the treatment of acute infections of *P. gallinaceum*, sulphadiazine is a more potent agent for the production of resistance to paludrine than is that drug itself. Sulphadiazine is not a drug commonly used in the treatment of human malaria, though it has been shown to have an effect upon these parasites (Coggeshall, Maier & Best, 1941; Leishman & Kelsall, 1944), and its suppressive action, particularly upon infections of *P. falciparum*, has been demonstrated (Fairley, 1945); it is, however, of great importance in the treatment of bacterial infections. It would be unwise to assume that results obtained from experiments upon acquired resistance to drugs in avian malaria can be applied directly to human malaria, and that the use of sulphadiazine in the treatment of intercurrent bacterial infections in patients suffering from malaria might give rise to paludrine-resistant strains of the malaria parasite; but the possibility cannot be ignored. Our preliminary experiments have shown that when latent infections of *P. gallinaceum* are treated with sulphadiazine, paludrine resistance is not induced within the period of treatment (75 days). If cross-resistance between sulphadiazine and paludrine should be proved to occur in human malaria, then, by analogy with avian malaria, the danger of producing paludrine-resistant strains by the use of sulphadiazine in the treatment of patients carrying latent infections of malaria should be slight; it would be in its use during acute attacks of malaria that the danger of establishing paludrine-resistant strains might arise.

In the development of resistance both to paludrine and sulphadiazine we have observed that strains of *P. gallinaceum* may become resistant to higher concentrations of the drugs than those to which they have been exposed. Thus strain C (Table 5), which had been treated with 0·625 mg. doses of sulphadiazine, became resistant to 5 mg. doses of the drug and later produced low-grade infections of normal parasites in birds receiving 20 mg. doses of the drug. Likewise, a strain of *P. gallinaceum* treated with 0·1 mg. doses of paludrine (p. 172) became fully resistant to doses of 0·5 mg. It must be remembered that the amounts of drug refer to the quantities administered to the host; we have, in the course of this work, made no estimations of the concentration of drug present in the plasma and erythrocytes and therefore in direct

contact with the parasites. Our observations suggest that the intensity of resistance to these drugs may continue to develop although the dosage of drug is not increased. As already stated, however, when a strain of *P. gallinaceum* is treated with sulphadiazine, resistance to paludrine appears at once to extend to the full range of doses tolerated by the chick, though growth in the higher doses of paludrine is at first slow.

In any attempt to explain the mechanism of cross-resistance between sulphadiazine and paludrine the following points must be considered. Resistance to paludrine is induced more rapidly by sulphadiazine than by paludrine itself, and strains of *P. gallinaceum* treated with sulphadiazine may become highly resistant to paludrine before resistance to sulphadiazine can be detected. But, whereas resistance to paludrine is induced readily by the treatment of patent infections of *P. gallinaceum* with sulphadiazine, the converse is not true; up to the present, resistance to sulphadiazine has been induced by treatment with paludrine in one strain only and after prolonged treatment with large doses of the drug.

In becoming resistant to sulphadiazine *P. gallinaceum* does not thereby become resistant to all compounds having antimalarial action. Resistance as induced by sulphadiazine has been shown to extend to related sulphonamides and to paludrine and its methyl homologue M 4430; it does not extend to quinine, mepacrine or pamaquin, indeed its sensitivity to this latter compound is increased. This might suggest that the mode of action of paludrine, M 4430 and the sulphonamides is similar, and different from that of quinine, mepacrine and pamaquin.

It has been shown by Maier & Riley (1942) in *P. gallinaceum* and by Marshall, Litchfield & White (1942) in *P. lophurae* that the antimalarial action of sulphonamides is inhibited by *p*-aminobenzoic acid—an observation which we have confirmed upon our normal strain of *P. gallinaceum* (Bishop & McConnachie, 1948). The mechanism of action of sulphonamides upon malaria parasites therefore appears to be similar to that generally accepted for bacteria. If cross-resistance between sulphadiazine and paludrine were due to a straightforward similarity in mode of action between the two drugs, we should expect not only that it would be induced by either drug with equal ease, but also that the antimalarial action of paludrine, like that of sulphadiazine, would be antagonized by *p*-aminobenzoic acid. Preliminary experiments, however, have shown that the action of paludrine is not reversed by *p*-aminobenzoic acid (Bishop & McConnachie, 1948). In these experiments groups of chicks were treated with 0·1 mg. doses twice daily of paludrine, or similar doses of *p*-aminobenzoic acid for 3½ days, or with the two drugs successively. No significant difference was observed in the intensity of the infections which developed in the group of chicks treated with paludrine alone and that treated with paludrine and *p*-aminobenzoic acid. Terzian, Stahler & Weathersby (1949), in a recent study of the effect of antimalarial drugs upon the development of *P. gallinaceum* in mosquitoes, have found that whereas *p*-aminobenzoic acid inhibits the action of sulphadiazine upon the developing oöcysts it is without effect upon paludrine. It must be concluded, therefore, that the antimalarial action of sulphadiazine which is antagonized by *p*-aminobenzoic acid is not common to paludrine.

In considering this problem further, two recent observations upon the action of these drugs are of importance; one deals with potentiation between paludrine and sulphadiazine, and the other with the indirect action of paludrine. Greenberg, Boyd & Josephson (1948) have found that when paludrine and sulphadiazine are given concurrently the action of each is potentiated; thus the effective dose of paludrine could be reduced to one quarter if it was given with $\frac{1}{32}$–$\frac{1}{64}$th part of the effective dose of sulphadiazine. It was not possible to explain this potentiation effect in terms of increased plasma concentrations of the drugs. Hawking & Perry (1948*a*) found that paludrine itself is not active upon parasites of *P. cynomolgi* and *P. gallinaceum in vitro*, but becomes changed into an active compound in the body of the host. It can be activated also *in vitro* by exposure to minced liver cells. The concentrations of paludrine used in the *in vitro* tests were higher than those found in the blood during normal dosage. In contact with the body cells, therefore, paludrine undergoes some chemical change which converts it from an inactive into an active compound.

The problem of cross-resistance between sulphadiazine and paludrine, therefore, is complicated by this 'activation' of paludrine, and further research, both upon the constitution of the active compound, and upon the relationship of paludrine to sulphadiazine and to other sulphonamides is required before any solution can be hoped for.

10. SUMMARY

1. A thirty-two-fold increase in resistance to sulphadiazine has been induced in *Plasmodium gallinaceum* in chicks by treatment with that drug.

2. No loss in resistance to sulphadiazine occurred in the resistant strain during cyclical passage through *Aëdes aegypti*.

3. The sulphadiazine-resistant strain was resistant also to sulphathiazole, sulphanilamide and sulphapyridine, but not to mepacrine, quinine or pamaquin. An increase in sensitivity to pamaquin was observed.

4. The sulphadiazine-resistant strain was resistant to paludrine and its methyl homologue M 4430.

5. In strains maintained in a state of acute infection and treated with sulphadiazine, resistance to paludrine developed more rapidly than resistance to sulphadiazine, and in one strain a high degree of resistance to paludrine was obtained before any increase in resistance to sulphadiazine could be detected.

6. Resistance to paludrine as induced by sulphadiazine, develops rapidly and extends at once over the full range of doses which the chick will tolerate, whereas resistance to paludrine as induced by that drug itself, develops more slowly and by stages.

7. Whereas resistance to paludrine is induced readily by treatment with sulphadiazine, resistance to sulphadiazine is induced by paludrine only after treatment with high doses of the drug for a prolonged period.

8. In latent infections of *Plasmodium gallinaceum* resistance to paludrine or sulphadiazine was not induced by sulphadiazine during the period of the experiment (49–75 days), though the aggregate dosage of drug used was much greater than that with which resistance was induced in strains maintained in an acute state.

9. It is not considered probable that cross-resistance between sulphadiazine and paludrine is due to a similar mode of action of these drugs, as whereas sulphadiazine is antagonized by *p*- aminobenzoic acid paludrine is not.

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CROSS-RESISTANCE BETWEEN SULPHANILAMIDE AND PALUDRINE (PROGUANIL) IN A STRAIN OF *PLASMODIUM GALLINACEUM* RESISTANT TO SULPHANILAMIDE

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1. INTRODUCTION

It has been shown recently that strains of *Plasmodium gallinaceum* resistant to paludrine (proguanil) can be developed by the treatment of patent infections in chicks with that drug (Bishop & Birkett, 1947, 1948; Williamson, Bertram & Lourie, 1947; Williamson & Lourie, 1947), and that strains resistant to sulphadiazine can likewise be obtained by the treatment of similar infections with sulphadiazine (Bishop & Birkett, 1948; Bishop & McConnachie, 1948). Moreover, it has been shown that if strains of *P. gallinaceum* are treated with small but effective doses of sulphadiazine whilst being maintained in a state of acute infection, they become resistant to paludrine and its methyl homologue, M4430, and that resistance to these drugs may develop before resistance to sulphadiazine can be detected (Bishop & McConnachie, 1948, 1949). In strains treated with paludrine, however, resistance to sulphadiazine has been obtained in one strain only and after prolonged treatment with large doses of the drug.

In *P. gallinaceum*, as in bacteria, the action of sulphadiazine is antagonized by *p*-aminobenzoic acid, whereas the antimalarial action of paludrine is unaffected by that compound. In attempting to understand the mechanism of cross-resistance between these two important drugs it is necessary to discover whether cross-resistance between sulphadiazine and paludrine is restricted to these compounds, or whether other sulphonamides antagonized by *p*-aminobenzoic acid also confer resistance to paludrine.

2. METHODS

Sulphanilamide was chosen, in the first instance, for comparison with sulphadiazine, as it is the simplest in structure of all the sulphonamides antagonized by *p*-aminobenzoic acid. The method used to develop a strain of *P. gallinaceum* resistant to this drug was similar to that used in our earlier work (Bishop & Birkett, 1948). A substrain of the normal parent strain, which at no time had been in contact with drugs and which had recently been proved to retain a normal sensitivity to paludrine, was maintained throughout the period of drug treatment, in chicks 45–65 g. in weight, by intravenous passage at intervals of 2–4 days. The inocula varied in size from 0·1 to 0·5 c.c. of heavily infected, heparinized blood. By this method the strain remained in a state of acute infection throughout the period of treatment with sulphanilamide. The drug was given orally in aqueous solution. During the first few passages† 2 mg. of sulphanilamide was given once daily on consecutive days, with the exception of Sundays, when no drug was given. As this dose had little apparent effect upon the parasites or upon the intensity of infection, twice-daily doses of 2 mg. were given. After several passages through birds receiving 2 mg. twice daily the dose was increased to 4 mg. twice daily, which had, at first, a marked effect upon

* Member of the Scientific Staff of the Medical Research Council.

† Throughout this account the dosage of drug is expressed in mg./20 g. of body weight. Unless stated otherwise the doses were given twice daily.

the parasites. After several passages through birds receiving 4 mg. twice daily the strain became adjusted to the drug and heavy infections of parasites normal in appearance were produced in the birds receiving this dose. Later 8 mg. twice daily was given, this being the maximum dose which it was possible to give, as, with this amount of drug, loss of weight occurred in the majority of birds. During the first few passages through birds receiving this increased dosage, many abnormal parasites were again observed, but gradually the proportion of abnormal to normal parasites grew smaller, and heavy infections of parasites, indistinguishable morphologically from those of the normal parent strain, were observed. As in the case of strains resistant to paludrine and sulphadiazine (Bishop & Birkett, 1948; Bishop & McConnachie, 1949), exo-erythrocytic parasites developed in birds undergoing treatment with the drug.

3. THE EFFECT OF PALUDRINE (PROGUANIL), SULPHATHIAZOLE, SULPHAPYRIDINE AND SULPHADIAZINE UPON A STRAIN OF *PLASMODIUM GALLINACEUM* RESISTANT TO SULPHANILAMIDE

After a strain of *P. gallinaceum* had been treated for 63 days with doses of sulphanilamide increasing from 2 mg. once daily to 4 mg. twice daily, its resistance to this drug, and also to paludrine and sulphadiazine, was tested by the method outlined above. Doses of 4 mg. of sulphanilamide were found to inhibit the growth of the sulphanilamide-treated strain less than that of the normal strain (Table 1). No growth of the sulphanilamide-treated strain occurred in birds treated with 1·25 mg. of sulphadiazine, but in birds treated with 0·1 mg. of paludrine, a dose which inhibited the development

Table 1. Plasmodium gallinaceum. *The effect of paludrine, sulphathiazole, sulphapyridine and sulphadiazine upon a strain resistant to sulphanilamide*

Total dosage of S.A.*	Duration of treatment in days	No. of passages	Maximum dose of S.A.*	Strain	Control	Paludrine			Sulphanilamide			Sulphadiazine	Sulphathiazole		Sulphapyridine	
						0·1*	0·5	1·0	2	4	8	1·25	5	10	5	10
278	63	17	4	Resistant	378	299	—	—	353	194	—	1	—	—	—	—
				Normal	389	1	—	—	256	51	—	14	—	—	—	—
322	70	18	4	Resistant	381	326	281	54	—	279	—	34	262	—	317	182
				Normal	234	0	—	—	—	22	—	2	39	—	103	—
2334	248	54	8	Resistant	339	—	—	—	—	—	347	167	—	222	—	321
				Normal	424	—	—	—	—	—	15	43†	—	25	—	40

* The dose of drug is given in mg./20 g. of body weight of bird. S.A. = sulphanilamide. The intensity of infection is expressed as the average number of infected erythrocytes per 500 erythrocytes in groups of 3–6 birds.

† The high count was due to the high rate of infection in one bird which presumably failed to absorb the drug.

The sulphanilamide-treated strain was tested for resistance to sulphanilamide and other sulphonamides and to paludrine by the method already described (Bishop & Birkett, 1947, 1948). Groups of 3–6 birds were inoculated intravenously with approximately 50,000,000 parasites of the sulphanilamide-treated strain per bird. One group of chicks remained untreated and served as controls, whereas the others were treated with the drugs to be tested. One dose of the drug was given immediately before inoculation and two doses were given on each of the following 3 days. The intensity of infection produced in the groups of chicks inoculated with the sulphanilamide-treated strain was compared with that of similarly treated groups inoculated with the normal parent strain, the intensity of infection being assessed on the 4th day following inoculation, as the number of infected erythrocytes per 500 erythrocytes.

of the normal strain completely, heavy infections were obtained. Although the strain had as yet acquired no marked degree of resistance to sulphanilamide, and none to sulphadiazine, it was definitely resistant to paludrine.

After treatment for a further 5½ days with 4 mg. of sulphanilamide twice daily, the resistance of the strain to sulphanilamide, sulphadiazine and paludrine was tested again (Table 1). In birds treated with 4 mg. doses of sulphanilamide the growth of the sulphanilamide-treated strain was much heavier than that of the normal parent strain. Slight infections of normal parasites were obtained in birds treated with 1·25 mg. of sulphadiazine. In birds treated with sulphathiazole* and sulpha-

* Thiazamide sodium (May & Baker), the soluble form of sulphathiazole, was used in these tests, and the amount of drug administered gave the dosage of sulphathiazole stated.

pyridine the infections produced by the sulphanilamide-treated strain were definitely heavier than those produced by the normal parent strain. In birds treated with paludrine heavy infections were obtained not only in birds treated with 0·1 mg. but also in those treated with 0·5 mg., and slighter infections occurred in birds treated with 1·0 mg. The sulphanilamide-treated strain had therefore become highly resistant to paludrine. After treatment twice daily with 8 mg. doses of sulphanilamide for a further period of 178 days, the reaction of the strain to the sulphonamide compounds was tested again. The growth of the sulphanilamide-treated strain in birds receiving 8 mg. doses of this drug twice daily was very heavy, whereas that of the normal parent strain was inhibited almost completely. The strain had, therefore, become definitely resistant to sulphanilamide. Growth of the sulphanilamide-resistant strain was good also in birds treated with sulphathiazole, sulphapyridine and sulphadiazine in doses which had a definite effect upon the growth of the normal strain. Resistance to sulphanilamide, therefore, conferred resistance to sulphathiazole, sulphapyridine and sulphadiazine in addition to a high degree of resistance to paludrine.

4. DISCUSSION

The antimalarial action of paludrine and the four sulphonamides (sulphadiazine, sulphathiazole, sulphapyridine and sulphanilamide) used in the foregoing experiments differs considerably in potency. Sulphadiazine is well tolerated by chicks, doses as great as 20 mg. twice daily being without effect upon the birds, and, as it inhibits the growth of *P. gallinaceum* in doses as small as 0·625–1·25 mg. twice daily, its chemotherapeutic index is considerable. Paludrine is more toxic to chicks than sulphadiazine; thus doses of 1·5 mg. twice daily, when given over several days, produce a marked loss in weight in the majority of birds. The minimum effective dose of paludrine is, however, very much smaller than that of sulphadiazine, and doses of 0·1 mg., when given twice daily for several days, suppress the development of the parasites in the peripheral blood completely, though they do not sterilize the birds of their infections. The suppressive action of paludrine is much more complete than that of sulphadiazine; in birds treated with sulphadiazine, even in doses as large as 5 mg., parasites are not infrequent in the peripheral blood though the majority of them are abnormal in appearance. Sulphathiazole and sulphapyridine have a definite antimalarial action only in doses of 10 mg. twice daily, and larger amounts of these drugs, when given over a period of days, lead to a marked loss of weight in the birds. The chemotherapeutic index of sulphanilamide is small, as doses of less than 4 mg. twice daily are without effect upon the intensity of infection, and doses of 8 mg. are not well tolerated by the birds.

In testing a strain of *P. gallinaceum* resistant to sulphanilamide against sulphadiazine and paludrine we are, therefore, testing a strain resistant to a compound of low antimalarial potency against compounds of high potency. The development of cross-resistance to sulphadiazine, even in low doses, was slow, and the infections produced in birds receiving 1·25 mg. doses of that drug were not heavy even after the strain had been passaged through birds receiving 8 mg. doses of sulphanilamide for a long period. It is, therefore, all the more striking that cross-resistance to paludrine, a compound of even greater antimalarial potency than sulphadiazine, should develop so rapidly and that the degree of resistance shown should be so great. In an earlier publication (Bishop & McConnachie, 1949) we have shown that in a strain of *P. gallinaceum* treated with doses of 0·625 mg. of sulphadiazine twice daily, resistance to paludrine developed before resistance to sulphadiazine itself could be detected. These results together with those from the sulphanilamide-resistant strain suggest that paludrine acts upon some mechanism in the malaria parasite which is extremely sensitive to the action of sulphonamides of the type which we have studied.

Cross-resistance to paludrine is not specific to sulphadiazine but extends to sulphanilamide, as has been proved by our experiments with the sulphanilamide-resistant strain of *P. gallinaceum*. As strains resistant to sulphadiazine and sulphanilamide are resistant likewise to sulphapyridine and sulphathiazole, it can be presumed that these drugs also would confer resistance to paludrine. Para-aminobenzoic acid competitively inhibits the bacteriostatic action of these sulphonamides. The ability of sulphonamides of this group to confer resistance to paludrine, in *P. gallinaceum*, is therefore remarkable as *p*-aminobenzoic acid is without effect upon paludrine (Bishop & McConnachie, 1948).

5. SUMMARY

1. Resistance to sulphanilamide, of a low order, has been developed in a strain of *Plasmodium gallinaceum* by the treatment of patent infections with the drug.

2. The sulphanilamide-resistant strain was resistant to paludrine over the whole range of doses tolerated by the host; it also proved to be resistant to sulphathiazole, sulphapyridine and sulphadiazine, though the degree of resistance shown was much slighter than to paludrine.

3. Resistance to paludrine developed in the sulphanilamide-resistant strain before any resistance to sulphadiazine could be detected.

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THE REACTIONS OF *LUCILIA SERICATA* (Mg.) TO VARIOUS SUBSTANCES PLACED ON SHEEP

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INTRODUCTION

In a previous publication (Cragg & Ramage, 1945) it was shown that ethyl mercaptan in conjunction with ammonium carbonate was a powerful attractant for the blowfly *Lucilia sericata* (Mg.) when placed on living sheep. The hypothesis was advanced that under natural conditions sulphur compounds of that type might arise from the breakdown of cystine contained in fleece keratin and thus render the sheep susceptible to attack by the blowfly. Further experimental work has been carried out which extends the previous observations. In particular, attention has been given to the separation of the stimuli responsible for causing attraction and oviposition, a distinction in the oviposition response first made by Hobson (1938).

The present paper is confined to experiments carried out on sheep, but the problem of inducing attraction and oviposition without the living sheep, has been simultaneously studied (see Cragg & Thurston, 1949).

1. METHODS

The initial experiments were made during the summer months of 1944 on a flock of Welsh mountain sheep at Aber, Caernarvonshire. In 1947 the trials were repeated on a flock of Mule × Suffolk lambs at Houghall Farm, Durham, and supplementary observations have been obtained for Swaledale × Herdwick lambs at Crag Farm, Ravenglass, Cumberland. The sheep were numbered, used in batches of 6–10, and the precautions listed by Cragg & Ramage (1945) were again observed. The suitability of weather conditions for oviposition was usually tested at the beginning, and in certain cases at the end of each experiment. In the early experiments, conditions were tested by placing pads of 2 % ammonium carbonate and 0·04 % indole in the fleeces of two sheep. As soon as oviposition was recorded, the pads were removed and the two sheep moved from the experimental flock. This prevented the controls from exerting undue attraction on the fly population, and also prevented them from drawing flies within the orbit of the other sheep undergoing tests. In later experiments the attractant pads on control sheep were placed in open 3 × 1 in. glass tubes tied into the fleece. This prevented any 'hang-over' effect through the attractant contaminating the fleece.

At Aber the sheep during the tests were placed in a small enclosure approximately 0·5 acre, and at Ravenglass in an intake several acres in size. Conditions were much more uniform at the Durham centre. There, the experimental sheep were kept in poultry pens approximately 30 × 10 yards. There were twenty pens in all, arranged in two blocks of ten with a pathway approximately 3 yards wide running between the blocks. It was therefore possible to have trapping experiments and tests on sheep running simultaneously under very similar conditions.

2. ADDITION OF CYSTINE TO THE FLEECE

The following trials were made to determine whether cystine (solid) or cysteine hydrochloride solution placed in sheep fleeces led to oviposition.

Exp. 1. In each of four Welsh sheep (North Wales, 1944) 0·25 g. of cystine was dusted into an area approximately 6 sq.cm. Another group had areas of similar size marked on them, but with no cystine added. The selected areas in both groups were moistened daily for a period of 8 days. Oviposition

was not recorded on either group, although intervals suitable for fly activity did occur during the period.

Exp. 2. Negative results were also obtained in a similar trial on Welsh sheep using a saturated solution of cysteine hydrochloride in place of cystine.

Exp. 3. 0·5 g. of cystine was dusted into the shoulder and rump regions of five lambs of Mule × Suffolk (Durham, 15 June 1947). In the control group, oviposition was recorded on three sheep after an interval of 30, 31 and 33 days respectively. In the experimental group, oviposition on the shoulder region of all five sheep was noted 8, 9, 31 and 33 (2) days after dusting.

Exp. 4. At Ravenglass, Cumberland, 0·5 g. of cystine was dusted into the shoulder region on each of twenty Swaledale × Herdwick lambs. Another twenty were marked as controls but not treated in any way. During the period August–September no strikes were recorded in either group, although conditions suitable for strike did occur on occasions.

It would appear, from these experiments, that the presence of free cystine in a sheep's fleece did not increase the chance of oviposition.

3. ATTRACTANT PROPERTIES OF SOME ORGANIC SULPHUR COMPOUNDS

In previous studies on the attraction of blowflies to sheep it has been usual to follow the method first used by Hobson (1935) of measuring attraction in terms of the number of egg batches laid in the vicinity of the attractant. Thus a substance designated as an 'attractant' has in actual practice meant one which was able to induce oviposition. The fact that substances labelled attractants could, when used apart from sheep, attract blowflies to traps, without necessarily inducing oviposition (Cragg & Ramage 1945), obviously led to some confusion in the use of the term. The confusion was increased when in the course of the present investigation it was clear that, even on the sheep, attraction of the fly and oviposition by it, were not necessarily induced by the same substance. It is advisable, therefore, to confine the use of the term attractant to a substance which attracts flies although not necessarily inducing oviposition, and it is in this sense that the term is used in the present publication.

Table 1 lists compounds, all of which might be considered as breakdown products of cystine; these were tested as attractants by a variety of procedures. In some cases straight comparative tests were made between 3–5 sheep carrying one pad each of one substance and a similar number carrying one pad each of another substance. In other trials 2–4 pads were placed on individual sheep.

In 1944, using Welsh sheep, each of the liquid compounds listed in Table 1 (with the exception of dimethyl disulphide) was given a minimum of nine trials under conditions when oviposition could be induced on control sheep. Ethyl mercaptan proved to be the most powerful as an attractant. Oviposition was never recorded on pads of these substances. Hydrogen sulphide used alone did not exhibit any power of attraction (see p. 183).

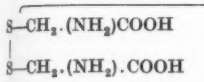
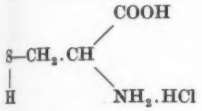
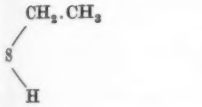
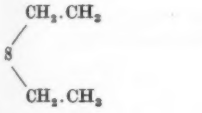
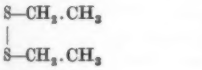
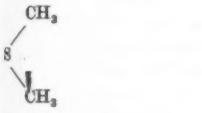
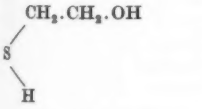
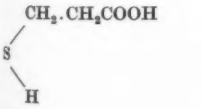
From previous experiments it was known that ethyl mercaptan, in conjunction with a concentration of ammonium carbonate which alone was inactive, gave a mixture capable of inducing oviposition. These experiments have been extended and, providing suitable weather conditions prevail, each of the liquid organic sulphur compounds listed in Table 1 would induce oviposition when mixed with 0·1 % ammonium carbonate or when a pad of that substance closely adjoined a pad of the sulphur compound.

Alone, a 5 ml. pad of 0·1 % ammonium carbonate was too weak to attract blowflies and induce oviposition as shown in three tests, each comprising six sheep (Welsh sheep, 1944), in which no attraction or oviposition occurred. When ethyl mercaptan pads (0·2 %) were added to half the group in each case, flies were attracted to such sheep, and oviposition occurred in the majority of cases. Oviposition only occurred on the ammonium carbonate pads. This experiment could be reversed providing the mercaptan pad was contained in a glass tube and contamination of the fleece prevented. Under these conditions, on sheep with ammonium carbonate and mercaptan pads, removal of the latter led to cessation of attraction.

That attraction and oviposition arose from two different sets of stimuli was emphasized by further experiments in which the mercaptan and ammonium carbonate pads were placed at varying distances apart. When the pads were 20–25 cm. apart, flies were attracted into the neighbourhood of the organic sulphur pad, rarely explored the fleece in the neighbourhood of the ammonium carbonate pad and no oviposition occurred (three trials). The closer the pads, the greater was the chance of the blowflies finding the ammonium carbonate, and when the distance was approx. 8–10 cm. oviposition invariably occurred (three trials).

The experiments described above were performed in 1944 on Welsh sheep in North Wales. During that blowfly season, forty tests were made in which ethyl mercaptan and ammonium carbonate pads were both placed on the same sheep and fifty-two tests using other related substances in place of ethyl mercaptan. In all these trials, oviposition was associated with the ammonium carbonate pad, and in no case did it occur on the pad containing the organic sulphur compound.

Table 1

Sulphur compounds			Concentration range tested (%)	Minimum concentration for oviposition in presence of ammonium carbonate (%)
	Cystine	Solid	Solid	—
	Cysteine hydrochloride	Solid	Saturated solution	—
	Ethyl mercaptan	37° C.	0·002–0·2	0·002
	Dimethyl disulphide	116° C.	0·001–0·2	not determined
	Diethyl sulphide	92° C.	0·002–0·2	0·02
	Diethyl disulphide	153° C.	0·001–0·2	0·02
	Dimethyl sulphide	38° C.	0·002–0·2	0·02
	β-hydroxy-ethyl mercaptan	52°/12 mm.	0·002–0·2	0·2
	β-mercaptan propionic acid	110°/15 mm.	0·002–0·2	0·2
	Hydrogen sulphide	Gas	Various concentrations (max. 0·3)	—

In 1947 the above tests were repeated on Mule × Suffolk lambs at Durham. In twenty-four trials using 0·02 % ethyl mercaptan and 0·1 % ammonium carbonate, oviposition was recorded on the ethyl mercaptan pads on three occasions. This happened only after the ammonium carbonate pads had been placed on the sheep, so that the experiments bore out the conclusions reached in 1944, that the oviposition stimulus was related to the presence of ammonium carbonate.

It was possible in 1947 to carry out a few tests with dimethyl disulphide. This appeared to be at least as powerful an attractant as ethyl mercaptan. It is evident from Table 2 that oviposition was mainly recorded on the dimethyl disulphide pads, but pads of 0·1 % ammonium carbonate were also present.

Table 2

	Pads	Occurrence of oviposition	No. of egg batches	Total no. of egg batches
A	0·02 % ethyl mercaptan	3	1–4	8
	0·1 % ammonium carbonate	6	1–4	16
B	0·02 % methyl disulphide	9	½–4	17½
	0·1 % ammonium carbonate	4	2–17	11+17

In each of twenty-four trials, pads making up the combinations A and B were placed on the same sheep in the rump region. On half the animals A was the anterior pair, on the remainder it was the posterior pair.

Table 3. *Comparative tests with ammonium compounds*

	No. of trials	Concentration tested (%)	No. in which oviposition occurred	No. of egg batches
		(*a*) Pads on different sheep		
Hydroxide	6	5·0	0	0
Carbonate	6	0·5	3	1
Carbonate	12	1·0	3	1
Bicarbonate	12	1·5	6	1–4
		(*b*) Pads of three substances per sheep		
Hydroxide	12	3·2 g. of ammonia/litre	0	0
Carbonate	12	ditto	4	1–2
Bicarbonate	12	ditto	10	1–7

4. TESTS WITH AMMONIUM COMPOUNDS

From the experiments described above, it was evident that the stimulus to oviposit was closely related to the presence of ammonium carbonate. From the earlier work of Hobson (1935) it was known that ammonium carbonate alone could, at concentrations of 0·5 % upwards, bring about attraction and oviposition, whilst ammonium hydroxide was not as active in producing oviposition.

In order to clarify the position as regards the power of ammonium compounds to produce oviposition, comparative tests were made with the hydroxide, carbonate and bicarbonate. Tests were made comparing two of these substances at one time (Table 3*a*).

In other trials in which the ammonium content was kept constant (solutions standardized by titration) ammonium hydroxide caused attraction of blowflies but no oviposition, whereas both the other substances could induce oviposition. Further, where the choice lay between bicarbonate and carbonate, the bicarbonate usually proved more active (see Table 3*b*).

These experiments clearly indicated that ammonia by itself was an attractant; but only in conjunction with carbon dioxide, as carbonate or bicarbonate, did oviposition occur. This conclusion was emphasized by the use of the two-pad technique, since 5 % ammonium hydroxide could be used as an attractant to ensure oviposition on a pad of 0·1 % ammonium carbonate.

5. THE ACTION OF CARBON DIOXIDE

Since comparative tests with ammonium salts had shown that carbon dioxide played a part in the stimulus which provoked oviposition, the action of carbon dioxide by itself, and in conjunction with other substances, was examined.

Solutions of carbon dioxide, prepared either by dissolving solid carbon dioxide in water or by bubbling the gas through water, were employed in the initial experiments.

Using the multiple-pad technique, the following

results were obtained. Alone, pads of carbon dioxide solution failed to provoke attraction or oviposition (twenty trials). In the presence of organic sulphur compounds which acted as attractants, no oviposition occurred (ten trials); but, in the presence of ammonium hydroxide of a strength sufficient to act as an attractant, oviposition occurred on both pads (two out of ten trials). On several occasions gravid females of *L. sericata* were observed moving between the two pads and at intervals probing the fleece with their ovipositors although oviposition did not take place.

When ethyl mercaptan was used as the attractant in the presence of pads of 0·2 % ammonium hydroxide (below the threshold of attraction) and carbon dioxide solution, oviposition was observed between the latter pads in three out of ten trials.

These experiments indicated that the oviposition stimulus was dependent on the combined presence of ammonia and carbon dioxide. Evidence that carbon dioxide could stimulate oviposition in the absence of ammonia was given in trials where pads of ethyl mercaptan were used as the attractant in the presence of pads of saturated sodium bicarbonate. Under conditions ideal for blowfly activity, oviposition was induced on the latter pad (six out of ten trials), but the number of egg batches was small, being one or two per sheep.

Several attempts were made under excellent conditions to induce oviposition by passing a stream of carbon dioxide into the fleece both in the presence and absence of ethyl mercaptan. In only one case, out of fifteen tests, were eggs laid, and they were scattered on the mercaptan pad. These experiments were carried out in a pen where sheep and flies could be closely observed. Under ideal conditions for blowfly activity, flies attracted to the sheep by the presence of mercaptan would explore the fleece in the neighbourhood of the carbon dioxide stream and test the site with their ovipositors, but the flies did not show the normal settling response which precedes oviposition. It is characteristic of flies about to oviposit that they become fairly resistant to disturbances in their neighbourhood. The wool can be moved quite roughly without the flies moving away. In the present case, although attracted to carbon dioxide areas produced near mercaptan pads, the flies readily moved away from them.

From these experiments it would appear that the most effective oviposition stimulus is a combination of ammonia and carbon dioxide.

6. THE IMPORTANCE OF HYDROGEN SULPHIDE

In the section on organic sulphur compounds it was pointed out that hydrogen sulphide solution alone was not an attractant, nor, in the presence of other attractants, did it stimulate oviposition. When combined with other sulphur compounds, however, it increased their power as attractants. Thus Cragg & Ramage (1945) showed that a solution containing 0·1 % ammonium carbonate and 0·001 % ethyl mercaptan, in itself unable to induce oviposition, could be made to do so by the addition of a trace of hydrogen sulphide. This adjuvant effect was further emphasized in the trapping experiments (Cragg & Thurston, 1949), where it was shown that ethyl mercaptan, or other related compounds, were relatively inactive as attractants unless used in conjunction with hydrogen sulphide.

In view of the low solubility of hydrogen sulphide in water, attempts were made to bring about attraction by directing a stream of the gas into the fleece. Attraction was never obtained in such experiments, although the addition of a pad of ethyl mercaptan to such sheep resulted in attraction.

The fact that sulphydryl compounds without hydrogen sulphide behaved as attractants on the living fleece whereas they showed little activity in trapping experiments unless hydrogen sulphide was added, suggested the possibility that the fleece atmosphere might contain hydrogen sulphide and thus provide its own sensitizing agent for the mercaptans, sulphides or disulphides.

Efforts were made to test this possibility by tying lead acetate papers in various parts of the fleece:

(*a*) Lead acetate papers were placed in the fleeces of six Welsh lambs. The papers on four of them showed a distinct mottling within 24 hr., and in all cases were brown after being in the fleece for 4 days.

(*b*) Papers placed in three positions (shoulder, side of neck, rump) on five Swaledale × Herdwick lambs showed no marked change after 7 days' exposure.

(*c*) Papers placed at two positions on Mule × Suffolk lambs showed no marked change after 7 days' exposure, with slight browning after 14 days' exposure.

(*d*) Papers were placed in two groups of five Mule × Suffolk lambs, one of which had been dipped with a commercial arsenic-sulphur preparation 4 days prior to the test. All the papers placed in the dipped sheep showed a distinct browning within 24 hr., whereas those placed in undipped sheep showed no more than a slight coloration after 7 days.

As controls, lead acetate papers were kept in samples of unwashed sheep wool. The changes in colour recorded above were not recorded in the control pieces even when such wool samples were exposed out of doors for periods as long as 4 weeks.

Laboratory experiments showed that unwashed sheep wool which had been stored in the laboratory for 5 months produced browning of lead acetate papers within 17 hr. when moist and incubated at 37° C. Samples kept dry at 37° C. or moist but

maintained at room temperature showed no change even after 70 hr. Further, this rapid darkening of the papers could be retarded by washing the wool samples prior to incubation. Thus, samples washed with hot soapy water produced no browning even after 7 days' incubation at 37° C., whereas samples merely washed in cold water produced a most intense discoloration of the papers.

It would appear from the above observations that hydrogen sulphide is sometimes present in the fleece atmosphere of living sheep, and therefore might sensitize other sulphur-containing compounds placed in the fleece.

7. THE INDOLE PROBLEM

In the present investigation attention was concentrated on studying the activity of the organic compounds and no analysis was made of the role of indole. In the course of the work, however, some trials were made which extend the observations recorded by Cragg & Ramage (1945). At that stage of the work, on the basis of trials quoted as Exp. 1 (see Table 4), and isolated trials with other concentrations of the three materials, it was concluded that, under the conditions existing at the time, ethyl mercaptan in conjunction with ammonium carbonate was more active in inducing oviposition than a similar concentration of indole with ammonium carbonate. From Table 4 it will be seen that in later trials indole proved more active than ethyl mercaptan. On the data available it is not possible to state the conditions which influence the degree of activity of these compounds. The influence of weather conditions on the activity of these substances is not known.

DISCUSSION

From the results of this investigation it is clear that for sheep under field conditions some distinction can be made between the added stimuli which can attract *L. sericata* to sheep and those which will induce oviposition. Ammonia and various organic sulphur compounds enhanced attraction, but, for oviposition to occur, ammonia and carbon dioxide were necessary.

The difference between the two types of stimuli is not absolute. For example, indole provokes both attraction and oviposition although its activity is increased in the presence of ammonium carbonate (Hobson, 1936; Mackerras & Mackerras, 1944). Further, Hobson (1935) recorded oviposition on pads of 5% ammonium hydroxide, a response not obtained in the present investigation. It is conceivable that a substance which in a large number of trials only induces attraction may under more suitable climatic conditions produce oviposition.

The role of these various substances in the occurrence of oviposition under natural conditions is difficult to assess. Both ammonia and hydrogen sulphide have been shown to be present in materials which provoke oviposition under field conditions (Hobson, 1935; Cragg & Ramage, 1945). The present investigation clearly suggests the importance of two constituents of the fleece atmosphere, hydrogen sulphide and carbon dioxide. Hydrogen sulphide used alone showed no attractant properties when placed in the fleece of living sheep, but it had the power of increasing the activity of organic sulphur compounds. Whilst no data were obtained for the effect of carbon dioxide on such substances placed on sheep, trapping experiments (Cragg & Thurston,

Table 4. *Comparative trials with indole and ethyl mercaptan in the presence of ammonium carbonate*

			Indole			Ethyl mercaptan		
No.	Centre	No. of trials	Occurrence of oviposition	No. of egg batches	Total no. of egg batches	Occurrence of oviposition	No. of egg batches	Total no. of egg batches
1	North Wales,* Sept. 1943	9	0	0	0	5	1–3	9
2	North Wales, June 1944	12	0	0	0	5	1–2	8
3	North Wales, Aug. 1944	8	7	1–2	12	1	2	2
4	Cumberland, Aug. 1945	12	8	1–7	35	4	1–5	10
5	Durham, July–Aug. 1947	7	5	1–20	5+40	3	1–7	9

* Abstract of the results quoted in Cragg & Ramage (1945).

The concentrations compared in these experiments were:

(1) 0·1% ammonium carbonate with 0·01% of the other substance.
(2) 2·0% ammonium carbonate with 0·04% of the other substance.
(3) 0·1% ammonium carbonate with 0·02% of the other substance.
(4) 0·2% ammonium carbonate with 0·02% of the other substance.
(5) 0·1% ammonium carbonate with 0·02% of the other substance.

1949) showed that carbon dioxide increased the attractiveness of some organic sulphur compounds. Thus, both hydrogen sulphide and carbon dioxide may well increase the attractant properties of organic sulphur compounds produced in the fleece atmosphere.

The actual source of sulphur and ammonium compounds in the living fleece (excepting the conditions which occur on sheep which are scouring) is not definitely known. In a previous paper (Cragg & Ramage, 1945) it was suggested that they might arise from a breakdown of the cystine component of fleece keratin, and indirect evidence supported such an hypothesis. However, it has not been possible to produce attraction or oviposition by placing cystine in the fleece of living sheep, but the decomposition of such an added chemical may not have occurred under field conditions. There is therefore little support for possible bacterial or enzymatic action, apart from the detection of hydrogen sulphide in moist weathered sheep wool maintained at 37° C.

Studies which may have a bearing on this point have been made in New Zealand by Macfarlane (1942). He advances the view that materials are produced in the tip region of the wool fibre which under conditions of rain or high humidity can produce substances attractive to the sheep blowfly. The experimental evidence supporting this hypothesis is given in the following statement: 'The ovipositional preferences of *Lucilia sericata* were tested on wet samples from Merino fleeces. In a small range of experiments twice as many eggs were laid on tip wool as on proximal wool with fleece rot or scald accretions. Normal samples of proximal staple were ignored by gravid flies.' Whether these tests were carried out on sheep or merely by exposure of wool samples in the open or in laboratory conditions is not stated. This is a particularly unfortunate omission since 90 % of the strike records obtained in the section on field observations were caused by *Calliphora laemica* not *Lucilia sericata*.

An important observation by Mackerras & Mackerras (1944) should also be noted. They showed that under insectary conditions *L. cuprina* would oviposit on sheep which were kept moist. Flies did not show an immediate response to such sheep, for oviposition occurred approximately 2 hr. after the initial wetting. Such a time lag did not occur when pads of substances such as ammonium carbonate were placed on sheep. Thus, the observations clearly point to a liberation of some material in the fleece. Cragg & Ramage (1945) cited various laboratory studies which showed that weathering or irradiation could produce changes in the sulphur content of wool. It is likely that the presence of moisture would result in increased bacterial or enzymatic action at the fleece temperature with the ultimate liberation of hydrogen sulphide, sulphur compounds and carbon dioxide from the fleece components.

The serous exudate which often forms on the skin of sheep and sweat products such as suint may be alternative sources of attractant materials. Both contain protein, and suint is rich in potassium carbonate (Freney, 1940). The moisture content of the fleece may vary markedly even under relatively dry atmospheric conditions (Cragg & Davies, 1947; Davies, 1948). In particular, the shoulder region is specially prone to sweating as Davies (1948) has demonstrated, and this area is often the site of oviposition even when it is free from obvious contaminants such as faeces (MacLeod, 1943).

The behaviour of *L. sericata* under field conditions in Great Britain shows marked differences from that of the related species, *L. cuprina*, under insectary conditions in Australia. From the comprehensive results of Mackerras & Mackerras (1944) it is evident that *L. cuprina* is much more readily stimulated to oviposit than is *L. sericata*. Not only will they oviposit on moist sheep but oviposition has even been recorded on pads moistened with water, in the absence of other attractants.

Mackerras & Mackerras (1944) have independently reported conclusions in agreement with the views expressed by Cragg & Ramage (1945) that a factor provided by the living sheep, labelled by Hobson the '*S*' factor, is not essential for the production of oviposition. Their results also point to various chemicals being more powerful when placed on the living sheep than when used alone. In the light of the present investigation this difference in activity of the '*S*' factor may arise from the presence of traces of sulphur compounds, ammonia and carbon dioxide.

SUMMARY

1. The reactions, under field conditions, of *Lucilia sericata* (Mg.) to various substances placed on sheep are described.

2. Attempts to produce either attraction or oviposition by placing cystine or cysteine hydrochloride in the living fleece gave negative results.

3. Several organic sulphur compounds (see Table 1) of a type which might arise from the breakdown of cystine have been tested. All showed some power of attraction for *L. sericata*. Ethyl mercaptan and dimethyl disulphide were the most powerful as attractants, but none of these substances was able to induce oviposition.

4. Tests with ammonium compounds and carbon dioxide have shown that, at the concentrations used, ammonia acted as an attractant. For oviposition to occur carbon dioxide had to be present.

5. Hydrogen sulphide was detected as a constituent of the fleece atmosphere of certain sheep.

This substance, whilst having no attractive powers when used alone on sheep, increased the attractiveness of organic sulphur compounds.

6. Comparative tests with ammonium carbonate-indole and ammonium carbonate-ethyl mercaptan mixtures have shown marked variations in the relative powers of these preparations to induce oviposition.

7. A distinction is drawn between stimuli which attract *L. sericata* to sheep and those which induce oviposition. Ammonia and various organic sulphur compounds enhanced attraction, but both ammonia and carbon dioxide were necessary for oviposition. It is suggested that some of these materials may be produced under natural conditions from the breakdown of fleece keratin or by the bacterial decomposition of sweat and similar products in the fleece.

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THE REACTIONS OF BLOWFLIES TO ORGANIC SULPHUR COMPOUNDS AND OTHER MATERIALS USED IN TRAPS

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INTRODUCTION

Cragg & Ramage (1945) advanced the view that organic sulphur compounds along with other materials produced in the breakdown of fleece keratin might play an important part in the attraction of blowflies to sheep. A study of the reactions of *Lucilia sericata* (Mg.) to such compounds when placed on sheep has been described by Cragg (1949). The present publication gives an account of the reactions of blowflies to these and other materials when used alone under field conditions. Furthermore, attention has been paid to the distinction drawn by Cragg (1949) between stimuli causing attraction and those causing oviposition.

1. METHODS

Many of the trials were made on the same site as used in 1943, but additional experiments were carried out in 1945 at Ravenglass, Cumberland, and in 1947 the problem was again thoroughly investigated under the very different climatic conditions of the north of England at Houghall Farm, Durham. No marked differences were recorded in the activity of the various attractants at the three centres. The results quoted in the tables are mainly those obtained at Houghall. It was possible at that centre to carry out large numbers of tests simultaneously under very uniform conditions, and often at the same time as trials were being made on sheep. At Houghall, traps of the type described in Cragg & Ramage (1945) were placed approximately 8 yards apart, round the margins of the poultry runs (Cragg, 1949). This arrangement allowed eight traps per run.

In the majority of tests, experiments were conducted in two runs. In each test two substances or mixtures of substances were compared, alternate traps being baited with the same material. The duration of each test was largely dependent on the degree of blowfly activity. In the majority of cases the interval was 1½ hr., but occasionally it was increased to 2–3 hr. At the completion of one trial the same test substances were again compared, but the order of the traps was reversed in order to overcome as far as possible variations in the blowfly activityat the different trapping positions.

Apart from a few exceptions only females of the various blowfly species were captured when using the chemical attractants described in this paper. In the trapping records which follow, therefore, it should be noted that the designation *L. caesar* includes *L. illustris* and possibly *L. ampullacea*, since it is at present impossible to differentiate between the females of these species with any degree of certainty. From the records of male captures in meat traps at the North Wales centre, *L. caesar* predominated at the time of the experiments. At Durham, no meat traps were maintained, but evidence from egg batches laid by captured females would suggest that *L. caesar* predominated in the catches up to early August, with *L. illustris* predominating in the late August-September experiments.

In the earlier work it appeared possible that the type of trap used—a simple cylinder of mosquito netting—played a part in the mechanism of attraction. Flies attracted to such traps would alight on them and walk over the netting suface. It seemed likely that they might pick up traces of the attractant

through the meshes of the trap. Experiments were made comparing the effectiveness of such traps with glass traps of the type used by Freney (1937) in his chemotropic studies in Australia. Results of these comparative experiments showed that the mosquito-net surface was not essential for the activity of the attractants (Table 1). The greater efficiency of net traps was probably related to the larger area covered by the openings of such traps, and to the fact that flies could escape more readily from the mouth region of glass traps.

2. SULPHUR COMPOUNDS AS ATTRACTANTS

(*a*) *Organic sulphur compounds and hydrogen sulphide*

On the whole, the various organic sulphur compounds referred to in Cragg (1949) showed practically no powers of attraction when used alone (Table 2), but mixed with hydrogen sulphide they formed a series of mixtures of marked attractive powers (Table 3*a*, *b*).

Table 1. *Comparison of standard net trap with glass trap*

Date 1944	Exposure interval (hr. G.M.T.)	Standard net trap		Glass trap		
		L. caesar	*L. sericata*	*L. caesar*	*L. sericata*	
16 July	10·0–14·0	0	5	0	2	With fleece
23 July	12·0–14·0	—	—	35	0	With fleece
24 July	15·0–16·0	—	—	37	1	No fleece
30 July	15–16·30	18	0	0	0	No fleece
30 July	17·0–19·0	37	0	1	0	No fleece
6 Aug.	15·0–16·0	21	0	0	0	No fleece
6 Aug.	16·0–18·30	54	3	27	0	No fleece

The attractant used consisted of 10 ml. each of saturated hydrogen sulphide and 0·1 % ethyl mercaptan.

Table 2. *Attractant properties of individual organic sulphur compounds*

Date	Material	Total no. of tests	No. of tests in which no. of *Lucilia* species captured was		
			Nil	<5	>10
1944	Ethyl mercaptan	42	38	3	0
1947	Ethyl mercaptan	21	17	2	2
1947	Dimethyl disulphide	30	6	17	4
1944	Diethyl disulphide	24	21	3	0
1947	Diethyl disulphide	30	12	16	0
1944	Ethyl sulphide	24	24	0	0
1947	Methyl sulphide	12	12	0	0
1944	β-Hydroxy-ethyl mercaptan	24	24	0	0
1944	β-Mercaptan propionic acid	24	23	1	0

Only those tests are included in the above table in which a control baited with ethyl mercaptan and hydrogen sulphide caught more than ten *Lucilia* spp.

Table 3. *Combination of various organic sulphur compounds with hydrogen sulphide*

	Date	No. of tests	0·2 % diethyl disulphide		0·2 % ethyl sulphide		0·2 % β-hydroxy-ethyl mercaptan		0·2 % β-mercaptan propionic acid	
			L. caesar	*L. sericata*	*L. caesar*	*L. sericata*	*L. caesar*	*L. sericata*	*L. caesar*	*L. sericata*
(*a*) 1944	10 Sept.	4	68	0	18	0	—	—	—	—
	11 Sept.	2	49	0	26	0	0	1	1	1
	12 Sept.	6	60	6	—	—	1	1	—	—
	13 Sept.	4	—	—	18	0	—	—	0	0
			0·2 % ethyl mercaptan		0·2 % dimethyl disulphide		0·2 % diethyl disulphide		0·2 % methyl disulphide	
(*b*) 1947	28 Aug.	8	222	10	249	21	—	—	—	—
	28 Aug.	8	52	16	—	—	—	—	4	1
	29 Aug.	8	25	1*	—	—	—	—	5	0
	26 Aug.	4	234	17	—	—	61	5	—	—
	26 Aug.	4	61	1*	—	—	92	5	—	—
	27 Aug.	4	20	0	—	—	45	7	—	—
	27 Aug.	4	89	10	—	—	8	7	—	—

* Ethyl mercaptan used at 0·02 % instead of 0·2 %.

Of the compounds tried, ethyl mercaptan and dimethyl disulphide formed the most effective mixtures with hydrogen sulphide. Furthermore, these two materials, ethyl mercaptan and dimethyl disulphide, were the only organic sulphur compounds which, when mixed together, formed a preparation comparable with that of ethyl mercaptan and hydrogen sulphide. The possibility cannot be ruled out that the dimethyl disulphide had hydrogen sulphide present as a contaminant in its manufacture.

Detailed trials were made using various concentrations of ethyl mercaptan and hydrogen sulphide (see Table 4). Activity, of course, varied with weather conditions, but these tests showed that high concentrations of both constituents gave the highest catches, thus indicating that attraction was not dependent on a trace of one constituent sensitizing the other. Further, activity was greater when the two substances were mixed together than when they were placed in separate containers (see Table 5). This conclusion was emphasized by a series of experiments in which traps were placed close together in pairs, the two substances, hydrogen sulphide and ethyl mercaptan, being exposed under separate traps. Flies were fairly uniformly distributed between the two traps, but the total number of flies caught was very much less than the number captured in the control traps baited with a mixture of the two materials.

On the basis of such results, a mixture of 10 ml. 0·2 % ethyl mercaptan and 10 ml. freshly prepared saturated hydrogen sulphide solution was used as a standard. The superiority of this preparation over the ammonium carbonate, indole and hydrogen sulphide preparation used by Cragg & Ramage (1945) was shown by trials made on the same site as that used for the 1943 experiments (see Table 6).

(*b*) *The action of carbon dioxide*

Gaseous carbon dioxide slowly bubbled through ethyl mercaptan rendered the material attractive. This combination, however, was not as powerful an attractant as that produced when hydrogen sulphide

Table 4. *Activity of ethyl mercaptan-hydrogen sulphide mixtures*

(*a*) 10 ml. 0·2 % ethyl mercaptan + various concentrations of hydrogen sulphide

		Strength of hydrogen sulphide							
		100 %		10 %		1 %		0·1 %	
Date 1947	No. of tests	*L. caesar*	*L. sericata*	*L. caesar*	*L. sericata*	*L. caesar*	*L. sericata*	*L. caesar*	*L. sericata*
12 July	8	80	13	131	16	—	—	—	—
13 July	16	66	8	—	—	6	2	—	—
14 July	12	—	—	82	22	—	—	12	2

(*b*) 10 ml. conc. hydrogen sulphide + different concentrations of ethyl mercaptan

		Strength of ethyl mercaptan			
		0·2 %		0·02 %	
Date 1947	No. of tests	*L. caesar*	*L. sericata*	*L. caesar*	*L. sericata*
12 July	14	139	24	26	6

(*c*) Dilutions of the standard mixture (10 ml. conc. hydrogen sulphide + 10 ml. 0·2% ethyl mercaptan)

		Strength of mixture					
		100 %		10 %		1 %	
Date 1947	No. of tests	*L. caesar*	*L. sericata*	*L. caesar*	*L. sericata*	*L. caesar*	*L. sericata*
29 July	12	—	—	37	7	25	1
30 July	12	142	2	—	—	27	4

Table 5.

		10 ml. hydrogen sulphide mixed with 10 ml. ethyl mercaptan		Two solutions exposed in different containers	
Date 1947	No. of tests	*L. caesar*	*L. sericata*	*L. caesar*	*L. sericata*
20 June	4	11	4	2	0
21 June	4	57	12	4	1
24 June	4	12	5	8	1
25 June	8	192	58	39	5

was used in place of carbon dioxide (Table 7). The attractive properties of these materials were not due to volatilization of the ethyl mercaptan by the gas stream, because air bubbled through ethyl mercaptan did not form an attractant.

At various times, using the ethyl mercaptan-carbon dioxide preparation as a control, the two gases carbon dioxide and hydrogen sulphide were were added to ethyl mercaptan and tested against controls of either ethyl mercaptan-hydrogen sulphide or ethyl mercaptan-carbon dioxide. None of these materials formed attractive mixtures with ethyl mercaptan. Further, when added to the standard preparation (ethyl mercaptan-hydrogen sulphide) they reduced rather than increased its attractive properties.

Table 6. *Comparison of* 1943 *attractant* with the ethyl mercaptan + hydrogen sulphide preparation*

Date 1944	No. of tests	1943 preparation*		Ethyl mercaptan + hydrogen sulphide	
		L. caesar	*L. sericata*	*L. caesar*	*L. sericata*
		(*a*) Attractants surrounded by sheep wool			
24 July	3	3	1	97	2
26 July	3	0	0	62	3
26 Aug.	1	14	2	75	1
		(*b*) Attractants used alone			
3 July	3	2	0	136	4
9 Aug.	2	0	0	62	3
11 Aug.	2	0	0	64	12
13 Aug.	2	1	1	65	2
14 Aug.	2	1	0	127	8

* This consisted of 10 ml. 20% ammonium carbonate, 5 ml. 0·12% indole in one vessel and 10ml. (saturated) hydrogen sulphide in a separate vessel.

Table 7. *Activity of carbon dioxide or hydrogen sulphide bubbled through ethyl mercaptan*

Date		No. of tests	Carbon dioxide bubbling through ethyl mercaptan		Hydrogen sulphide bubbling through ethyl mercaptan	
			L. caesar	*L. sericata*	*L. caesar*	*L. sericata*
1945	8 Sept.	1	0	2	42	0
	10 Sept.	1	9	0	46	0
	11 Sept.	1	2	2	13	1
	12 Sept.	1	1	0	0	0
	13 Sept.	1	0	0	3	2
1947	9 July	3	4	20	16	5
	12 July	3	16	6	62	9
	13 July	4	14	1	—	—
	14 July	1	6	1	19	3
	15 July	3	4	4	20	0

tested together to see whether they formed an attractive mixture. On a few isolated occasions small numbers of *Lucilia* spp. were captured in traps baited with the two gases. The power of attraction was small and not obviously greater than that sometimes shown by hydrogen sulphide used alone.

Carbon dioxide also formed attractive mixtures with dimethyl disulphide and ethyl disulphide.

(*c*) *The action of sodium salts, ammonium compounds and indole*

Sodium carbonate (2%), sodium bicarbonate (saturated), ammonium carbonate (1–5%), ammonium hydroxide (5%) and indole (0·001–0·02%)

3. THE OCCURRENCE OF OVIPOSITION

At the end of each experiment the jars containing the attractant and a small tuft of sheep wool were examined for egg batches. In the case of organic sulphur compounds used either alone or in combination with hydrogen sulphide, egg batches were of such infrequent occurrence that their presence can be assumed to have been accidental. Thus it was concluded that such materials exposed under the conditions observed in the present experiments did not induce oviposition. From the experiments on sheep (Cragg, 1949) it was clear that ammonia and carbon dioxide were important agents in stimulating egg-laying. A number of trials was therefore arranged in which jars containing attractants plus

materials known to induce oviposition on sheep were placed on the ground approximately 2 ft. apart. The materials used were in duplicate, one set contained a small plug of cotton wool, the other a plug of washed sheep wool. Trials were made on very warm days when fly activity was great.

The results are listed in Table 8. Oviposition was only recorded in nineteen cases out of a total of 156 tests, and was always associated with the presence of indole. Oviposition occurred both on cotton wool and sheep wool. The amount of oviposition was small, rarely being more than a single batch of eggs in each test. Further, flies never settled in numbers on the materials in the manner in which they settled on sheep.

Twelve of the nineteen egg masses were reared and the species of fly determined with the following results: seven *L. illustris*, four *L. sericata*, one *L. illustris* mixed with *L. sericata*. These results contrast markedly with those obtained at the same time on sheep, where all the batches bred out gave rise to *L. sericata*.

4. TRAPS PLACED AT DIFFERENT HEIGHTS

In the various trapping experiments described above, *L. sericata* was usually outnumbered in the catches by *L. caesar*. These findings are in agreement with those described by Cragg & Ramage (1945). Thus, materials which on sheep attracted practically nothing but *L. sericata*, when placed on the ground mainly attracted *L. caesar*.

This difference in behaviour of *Lucilia* spp. may arise from a number of causes (see Discussion, p. 193). It was not possible to investigate this problem comprehensively, but experiments were made to see whether the proportion of species caught was affected by the height at which an attractant was exposed.

Alternate traps in one of the poultry runs were suspended from the wire-netting fence so that the attractant was 2 ft. above ground-level, the approximate height at which attractants were exposed when placed on sheep. Sheep in an adjacent pen had

Table 8. *Attempts to induce oviposition*

Date 1947	No. of tests	Attractant	Occurrence of oviposition: Cotton wool	Occurrence of oviposition: Sheep wool	Temperature (° C.)
22 July	28	Hydrogen sulphide	0	0	27–30
22 July	28	0·04 % indole	0	0	27–30
22 July	28	Ethyl mercaptan	0	0	27–30
14 Aug.	6	5 % ammonium hydroxide	0	0	29–34
14 Aug.	6	0·02 % indole + S.M.*	0	0	33–34
19 Aug.	6	0·02 % indole + S.M.*	0	0	—
19 Aug.	6	0·02 % indole + S.M.*	1	3	28–33
14 Aug.	6	0·04 % indole + S.M.*	0	4	34–23
16 Aug.	6	0·04 % indole + S.M.*	0	0	29–36
18 Aug.	6	0·04 % indole + S.M.*	3	3	27–32
18 Aug.	6	0·04 % indole	0	0	28–34
14 Aug.	6	0·02 % ammonium carbonate + S.M.*	0	0	33–34
16 Aug.	6	0·02 % ammonium carbonate + S.M.*	0	0	29–36
19 Aug.	6	0·2 % ammonium carbonate + 0·04 % indole	1	1	28–33
20 Aug.	6	0·2 % ammonium carbonate + 0·04 % indole	1	2	27–32

* S.M. = standard mixture consisting of 10 ml. hydrogen sulphide solution + 10 ml. ethyl mercaptan.

Table 9

Date 1947	No. of tests	Traps placed on the ground: *L. caesar*	Traps placed on the ground: *L. sericata*	Traps suspended 2 ft. above the ground: *L. caesar*	Traps suspended 2 ft. above the ground: *L. sericata*
24 July	6	63	31	4	2
24 July	8	24	17	15	0
28 July	3	6	14	5	0
28 July	3	25	13	2	0
28 July	4	11	7	6	2

attractants placed in their fleeces. It was impossible to make a quantitative determination of the flies visiting the sheep, but by careful observation it was possible to state whether or not *L. caesar* was among the flies visiting them. In these experiments, only on one occasion was a female of *L. caesar* observed on the sheep, and only *L. sericata* was reared from eggs laid on the sheep.

The general conclusion from these experiments (Table 9) is that small differences in height are not responsible for any major difference in the behaviour of the two species *L. sericata* and *L. caesar*. Fewer attractants. In those studies the traps were placed in open situations, and it might be concluded that *Lucilia* spp. predominated in the catches because other species only occasionally occurred in the trapping areas.

Catches made with meat baits at the North Wales centre have shown quite clearly that the chemical attractants were selecting from a mixed blowfly population in which *Calliphora* spp. were at times quite numerous. It was noted, however, that in late summer, as the *Calliphora* spp. became more numerous, they reacted to the chemical attractants

Table 10. *The reaction of* Calliphora *spp. to chemical attractants. Seasonal changes recorded at Aber, Caernarvonshire*

	Catch in a trap baited with meat								Catch in a trap baited with ethyl mercaptan + hydrogen sulphide							
	L. sericata		*L. caesar*		*C. vomitoria*		*C. erythrocephala*		*L. sericata*		*L. caesar*		*C. vomitoria*		*C. erythrocephala*	
Date 1944	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
12 Aug.	2	2	1	9	0	0	0	0	0	5	0	0	0	0	0	0
13 Aug.	1	24	8	29	13	38	0	10	0	2	0	38	0	0	0	0
26 Aug.	0	9	0	16	2	62	1	4	0	1	0	75	0	0	0	0
1 Sept.	1	7	4	152	16	20	2	2	0	2	2	151	0	0	0	1
15 Oct.	0	2	1	35	21	49	2	11	0	0	0	93	1	124	0	1
19 Oct.	0	0	0	30	7	44	0	13	0	0	0	16	3	51	0	15
20 Oct.	0	0	0	2	0	16	1	2	0	0	0	12	0	14	0	0
21 Oct.	0	0	0	13	3	73	1	14	0	0	0	4	0	10	0	8
22 Oct.	0	0	0	18	13	118	5	22	0	0	0	10	0	40	0	0

Table 11. *Fly catches recorded in the Pennines (Moor House, Westmorland)*

		L. sericata		*L. caesar*		*C. vomitoria*	
Date 1947	Altitude (ft.)	♂	♀	♂	♀	♂	♀
2 Sept.	1840	0	4	0	5	0	40
2 Sept.	2250	0	0	0	8	1	24
2 Sept.	2850*	0	8	0	3	0	17
3 Sept.	1840	1	68	0	19	1	129
3 Sept.	1840	0	0	0	0	1	253
3† Sept.	1840	25	325	1	2	6	189

* Highest point on Dun Fell

All the traps except 3† Sept. were baited with the standard attractant, trap 3† Sept. was baited with meat.

flies were trapped at 2 ft., but this difference was largely due to the fact that flies could escape more easily from suspended traps. The important point in the results is not the reduction in the numbers of flies caught, but the fact that at 2 ft. *L. caesar* was still the main species trapped, whereas on sheep in a neighbouring pen it was rarely present and was certainly outnumbered by *L. sericata*.

5. CLIMATE AND THE ACTION OF ATTRACTANTS

In the previous sections emphasis has been laid on the numbers of *Lucilia* spp. caught by the various

to almost the same extent as they did to meat baits. This change in reaction of *C. vomitoria* in particular is well shown in Table 10, which comprises a group of trapping catches typical of the changes recorded in August-October at the North Wales centre.

A number of isolated observations made in the Pennines confirms the fact that under certain conditions species other than *Lucilia* will react to the chemical attractants. These results are given in Table 11. It is also of interest to note that *L. sericata*, the species mainly responsible for myiasis in Great Britain, can occur in large numbers on exposed hill conditions in the Pennines.

6. DISCUSSION

The results described above show a certain amount of agreement with those obtained by Cragg (1949) using the same materials on living sheep. In both cases, when the organic sulphur compounds were activated they proved to be powerful attractants of female *L. sericata*. In neither case did they induce oviposition. For oviposition to occur, ammonia and carbon dioxide or indole had to be present. Whilst oviposition could readily be stimulated on sheep, it was of relatively rare occurrence when the various chemicals were used alone. It is possible that this difference in activity was related to the temperature at which the material was exposed; a view also advanced by Mackerras & Mackerras (1944), who obtained somewhat comparable differences for *L. cuprina* under insectary conditions.

There were two major differences in the activity of the organic sulphur compounds when used alone or on sheep.

First, on sheep, the individual compounds were attractive to *L. sericata*. Used apart from sheep they exerted little attraction unless they were mixed with carbon dioxide or hydrogen sulphide. Such preparations, especially those containing ethyl mercaptan or dimethyl disulphide were powerful blowfly attractants. It appears possible in the light of these observations that the immediate activity of organic sulphur compounds when placed on sheep was related to the presence of hydrogen sulphide and carbon dioxide in the fleece atmosphere.

Freney (1937) and Hepburn & Nolte (1943) emphasized the importance of sulphur-rich materials as attractants. Individual sulphur compounds, however, did not show marked attractive powers, although Freney pointed out that ethyl mercaptan attracted flies into the vicinity of traps without causing them to enter. In the light of the present investigation it is evident that organic sulphur compounds require the presence of other substances before they become active. Both carbon dioxide and hydrogen sulphide can bring about such activity, and these compounds are certainly present in meat baits or other preparations undergoing bacterial decomposition.

The second major difference was the fact that *L. caesar* rarely occurred on sheep carrying attractants, whilst it was the predominant species captured in traps baited with attractants. Several possible explanations for this difference in response by female *L. caesar* are ruled out as a result of various field observations. Exposure of attractants at sheep height instead of at ground-level did not alter the reaction of *L. caesar* to them. Quantity of fleece was also without effect on the reaction of the species, because on several occasions traps were exposed in the middle of whole clipped fleeces and *L. caesar* was captured in such traps. Fleece colour was unimportant, for *L. sericata* was the only species which responded to attractants placed on a black sheep. Further, it is unlikely that *L. caesar* was repelled by movements of sheep, because the flies remained active in the vicinity of traps when sheep and also human beings were moving about in the immediate neighbourhood.

It is possible that a 'live-sheep' factor may counteract the effect of an attractant placed on sheep. If this is so, then it must vary considerably because, as MacLeod (1943) and others have shown, there are areas in Great Britain where *L. caesar* does oviposit on sheep. Furthermore, it is evident from results described in this paper that the reactions of various species of blowfly to attractants is closely dependent on local climate, varying not only between centres but at one centre. Holdaway (1933) has studied the difference in response of *L. sericata* and *L. caesar* to meat traps under light and shade conditions, and Nicholson (1934) has described the influence of temperature on a number of species of blowfly including *L. sericata*. Practically nothing is known, however, of the combined effects of temperature and humidity on the activity of these species. Thus, before studying hypotheses involving living sheep or the existence of races within the species *L. caesar* (on available evidence such a possibility cannot be ruled out), the effect of climatic factors on the species should be thoroughly examined.

One aspect of the present investigation should prove of practical importance in future trapping studies on blowflies, particularly *Lucilia* spp. It is evident that ethyl mercaptan, or dimethyl disulphide mixed with hydrogen sulphide, form powerful attractants for females of *L. sericata* and *L. caesar*. Hitherto in field investigations meat has been used as the attractive medium. This was far from satisfactory, partly because it could not be standardized and partly because it showed no selective powers. The mixtures used above overcome these two objections and offer a means of standardizing field-trapping studies to an extent previously impossible. Already the materials have been used in population studies (Cragg & Hobart, unpublished work).

SUMMARY

1. Seven organic sulphur compounds likely to be produced in the breakdown of cystine have been tested under field conditions as blowfly attractants. Two of them, ethyl mercaptan and dimethyl disulphide, when mixed with hydrogen sulphide or carbon dioxide formed powerful attractants for females of the blowflies *Lucilia caesar* (and *L. illustris*) and *L. sericata*.

2. In the majority of trials 10 ml. 0·2% ethyl

mercaptan mixed with 10 ml. freshly saturated hydrogen sulphide solution was used as the control attractant. As a standard in field-trapping studies this material overcomes many of the disadvantages of meat baits.

3. Sodium carbonate, sodium bicarbonate, ammonium hydroxide, ammonium carbonate and indole, at various concentrations, did not activate the organic compounds. Furthermore, when added to the ethyl mercaptan-hydrogen sulphide preparation they did not increase its activity.

4. In the present experiments oviposition was rarely induced. When it did occur it was associated with the presence of indole.

5. *L. caesar* responded to attractants placed 2 ft. from the ground as well as to the same materials placed on the ground. Height alone, therefore, was not responsible for the fact that *L. caesar* did not respond to the attractants when they were placed on sheep.

6. The responses of blowflies to chemical attractants was linked with climatic conditions. Thus, under certain conditions *Calliphora* spp., particularly *C. vomitoria*, responded to these attractants.

7. The results obtained in the present investigation emphasize the attractive nature of sulphur-containing compounds and the possible importance of hydrogen sulphide and carbon dioxide as sensitizing agents of such compounds on sheep.

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